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PATENT APPLICATION

Combinations of Genes for Producing Seed Plants Exhibiting Modulated Reproductive Development

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BACKGROUND OF THE INVENTION

A flower is the reproductive structure of a flowering plant. Following fertilization, the ovary of the flower becomes a fruit and bears seeds. As a practical consequence, production of fruit and seed-derived crops such as grapes, beans, corn, wheat, rice and hops is dependent upon flowering.

Early in the life cycle of a flowering plant, vegetative growth occurs, and roots, stems and leaves are formed. During the later period of reproductive growth, flowers as well as new shoots or branches develop. However, the factors responsible for the transition from vegetative to reproductive growth, and the onset of flowering, are poorly understood.

A variety of external signals, such as length of daylight and temperature, affect the time of flowering. The time of flowering also is subject to genetic controls that prevent young plants from flowering prematurely. Thus, the pattern of genes expressed in a plant is an important determinant of the time of flowering.

Given these external signals and genetic controls, a relatively fixed period of vegetative growth precedes flowering in a particular plant species. The length of time required for a crop to mature to flowering limits the geographic location in which it can be grown and can be an important determinant of yield. In addition, since the time of flowering determines when a plant is reproductively mature, the pace of a plant breeding program also depends upon the length of time required for a plant to flower.

Traditionally, plant breeding involves generating hybrids of existing plants, which are examined for improved yield or quality. The improvement of existing plant crops through plant breeding is central to increasing the amount of food grown in the world since

the amount of land suitable for agriculture is limited. For example, the development of new strains of wheat, corn and rice through plant breeding has increased the yield of these crops grown in underdeveloped countries such as Mexico, India and Pakistan. Unfortunately, plant breeding is inherently a slow process since plants must be reproductively mature before selective breeding can proceed.

For some plant species, the length of time needed to mature to flowering is so long that selective breeding, which requires several rounds of backcrossing progeny plants with their parents, is impractical. For example, perennial trees such as walnut, hickory, oak, maple and cherry do not flower for several years after planting. As a result, breeding of such plant species for insect or disease-resistance or to produce improved wood or fruit, for example, would require decades, even if only a few rounds of selection were performed.

Methods of promoting early reproductive development can make breeding of long generation seed plants such as trees practical for the first time. Methods of promoting early reproductive development also would be useful for shortening growth periods, thereby broadening the geographic range in which a crop such as rice, corn or coffee can be grown. Unfortunately, methods for promoting early reproductive development in a seed plant have not yet been described. Thus, there is a need for methods that promote early reproductive development. The present invention satisfies this need and provides related advantages as well.

DEFINITIONS

As used herein, the term "transgenic" refers to a seed plant that contains in its genome an exogenous nucleic acid molecule, which can be derived from the same or a different plant species. The exogenous nucleic acid molecule can be a gene regulatory element such as a promoter, enhancer or other regulatory element or can contain a coding sequence, which can be linked to a heterologous gene regulatory element.

As used herein, the term "seed plant" means an angiosperm or a gymnosperm. The term "angiosperm," as used herein, means a seed-bearing plant whose seeds are borne in a mature ovary (fruit). An angiosperm commonly is recognized as a flowering plant. The term "gymnosperm," as used herein, means a seed-bearing plant with seeds not enclosed in an ovary.

Angiosperms are divided into two broad classes based on the number of cotyledons, which are seed leaves that generally store or absorb food. Thus, a monocotyledonous angiosperm is an angiosperm having a single cotyledon, and a

dicotyledonous angiosperm is an angiosperm having two cotyledons. Angiosperms are well known and produce a variety of useful products including materials such as lumber, rubber, and paper; fibers such as cotton and linen; herbs and medicines such as quinine and vinblastine; ornamental flowers such as roses and orchids; and foodstuffs such as grains, oils, fruits and vegetables.

Angiosperms encompass a variety of flowering plants, including, for example, cereal plants, leguminous plants, oilseed plants, hardwood trees, fruit-bearing plants and ornamental flowers, which general classes are not necessarily exclusive. Such angiosperms include for example, a cereal plant, which produces an edible grain cereal. Such cereal plants include, for example, corn, rice, wheat, barley, oat, rye, orchardgrass, guinea grass, sorghum and turfgrass. In addition, a leguminous plant is an angiosperm that is a member of the pea family (*Fabaceae*) and produces a characteristic fruit known as a legume. Examples of leguminous plants include, for example, soybean, pea, chickpea, moth bean, broad bean, kidney bean, lima bean, lentil, cowpea, dry bean, and peanut. Examples of legumes also include alfalfa, birdsfoot trefoil, clover and sainfoin. An oilseed plant also is an angiosperm with seeds that are useful as a source of oil. Examples of oilseed plants include soybean, sunflower, rapeseed and cottonseed.

An angiosperm also can be a hardwood tree, which is a perennial woody plant that generally has a single stem (trunk). Examples of such trees include alder, ash, aspen, basswood (linden), beech, birch, cherry, cottonwood, elm, eucalyptus, hickory, locust, maple, oak, persimmon, poplar, sycamore, walnut and willow. Trees are useful, for example, as a source of pulp, paper, structural material and fuel.

An angiosperm also can be a fruit-bearing plant, which produces a mature, ripened ovary (usually containing seeds) that is suitable for human or animal consumption. For example, hops are a member of the mulberry family prized for their flavoring in malt liquor. Fruit-bearing angiosperms also include grape, orange, lemon, grapefruit, avocado, date, peach, cherry, olive, plum, coconut, apple and pear trees and blackberry, blueberry, raspberry, strawberry, pineapple, tomato, cucumber and eggplant plants. An ornamental flower is an angiosperm cultivated for its decorative flower. Examples of commercially important ornamental flowers include rose, orchid, lily, tulip and chrysanthemum, snapdragon, camellia, carnation and petunia plants. The skilled artisan will recognize that the methods of the invention can be practiced using these or other angiosperms, as desired.

Gymnosperms encompass four divisions: cycads, ginkgo, conifers and gnetophytes. The conifers are the most widespread of living gymnosperms and frequently are

cultivated for structural wood or for pulp or paper. Conifers include redwood trees, pines, firs, spruces, hemlocks, Douglas firs, cypresses, junipers and yews. The skilled artisan will recognize that the methods of the invention can be practiced with these and other gymnosperms.

As used herein, the term "non-naturally occurring seed plant" means a seed plant containing a genome that has been modified by man. A transgenic seed plant, for example, is a non-naturally occurring seed plant that contains an exogenous nucleic acid molecule and, therefore, has a genome that has been modified by man. Furthermore, a seed plant that contains, for example, a mutation in an endogenous floral meristem identity gene regulatory element as a result of calculated exposure to a mutagenic agent also contains a genome that has been modified by man. In contrast, a seed plant containing a spontaneous or naturally occurring mutation is not a "non-naturally occurring seed plant" and, therefore, is not encompassed within the invention.

"Reproductive development" refers to the production of floral organs, including but not limited to sepals, petal, stamens, carpels as well as polen, ovules and/or seed. "Reproductive development" initiates upon the development of the floral meristem, typically derived from a shoot meristem.

The term "recombinant nucleic acid molecule," as used herein, means a non-naturally occurring nucleic acid molecule that has been manipulated *in vitro* such that it is genetically distinguishable from a naturally occurring nucleic acid molecule. A recombinant nucleic acid molecule of the invention comprises two nucleic acid molecules that have been manipulated *in vitro* such that the two nucleic acid molecules are operably linked.

As used herein, the term "inducible regulatory element" means a nucleic acid molecule that confers conditional expression upon an operably linked nucleic acid molecule, where expression of the operably linked nucleic acid molecule is increased in the presence of a particular inducing agent as compared to expression of the nucleic acid molecule in the absence of the inducing agent. In a method of the invention, a useful inducible regulatory element has the following characteristics: confers low level expression upon an operably linked nucleic acid molecule in the absence of an inducing agent; confers high level expression upon an operably linked nucleic acid molecule in the presence of an appropriate inducing agent; and utilizes an inducing agent that does not interfere substantially with the normal physiology of a transgenic seed plant treated with the inducing agent. It is recognized, for example, that, subsequent to introduction into a seed plant, a particularly

useful inducible regulatory element is one that confers an extremely low level of expression upon an operably linked nucleic acid molecule in the absence of inducing agent. Such an inducible regulatory element is considered to be tightly regulated.

The term "operably linked," as used in reference to a regulatory element, such as a promoter or inducible regulatory element, and a nucleic acid molecule encoding a floral meristem identity gene product, means that the regulatory element confers regulated expression upon the operably linked nucleic acid molecule encoding the floral meristem identity gene product. Thus, the term operably linked, as used herein in reference to an inducible regulatory element and a nucleic acid molecule encoding a floral meristem identity gene product, means that the inducible regulatory element is linked to the nucleic acid molecule encoding a floral meristem identity gene product such that the inducible regulatory element increases expression of the floral meristem identity gene product in the presence of the appropriate inducing agent. It is recognized that two nucleic acid molecules that are operably linked contain, at a minimum, all elements essential for transcription, including, for example, a TATA box. One skilled in the art knows, for example, that an inducible regulatory element that lacks minimal promoter elements can be combined with a nucleic acid molecule having minimal promoter elements and a nucleic acid molecule encoding a floral meristem identity gene product such that expression of the floral meristem identity gene product can be increased in the presence of the appropriate inducing agent.

As used herein in reference to a nucleic acid molecule of the invention, the terms "sense" and "antisense" have their commonly understood meanings.

As used herein in reference to a nucleic acid molecule of the invention, the term "fragment" means a portion of the nucleic acid sequence containing at least about 50 base pairs to the full-length of the nucleic acid molecule. In contrast to an active fragment, as defined herein, a fragment of a nucleic acid molecule need not encode a functional portion of a gene product.

The phrase "nucleic acid sequence" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role.

A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of an operably linked nucleic acid. As used herein, a "plant promoter" is a promoter that functions in plants. Promoters include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA

element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

The term "plant" includes whole plants, plant organs (e.g., leaves, stems, flowers, roots, etc.), seeds and plant cells and progeny of same. The class of plants which can be used in the method of the invention is generally as broad as the class of flowering plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), as well as gymnosperms. It includes plants of a variety of ploidy levels, including polyploid, diploid, haploid and hemizygous.

A polynucleotide sequence is "heterologous to" an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, a promoter operably linked to a heterologous coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from any naturally occurring allelic variants.

A polynucleotide "exogenous to" an individual plant is a polynucleotide which is introduced into the plant, or a predecessor generation of the plant, by any means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include Agrobacterium-mediated transformation, biolistic methods, electroporation, in planta techniques, and the like.

The phrase "host cell" refers to a cell from any organism. Preferred host cells are derived from plants, bacteria, yeast, fungi, insects or other animals. Methods for introducing polynucleotide sequences into various types of host cells are well known in the art.

The "biological activity of a polypeptide" refers to any molecular activity or phenotype that is caused by the polypeptide. For example, the ability to transfer a phosphate to a substrate or the ability to bind a specific DNA sequence is a biological activity. One

biological activity of of the gene products of the invention is the ability to modulate the time of development of reproductive structures in plants.

An "expression cassette" refers to a nucleic acid construct, which when introduced into a host cell, results in transcription and/or translation of an RNA or polypeptide, respectively. Antisense or sense constructs that are not or cannot be translated are expressly included by this definition.

In the case of both expression of transgenes and inhibition of endogenous genes (e.g., by antisense, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only "substantially identical" to a sequence of the gene from which it was derived. As explained below, these substantially identical variants are specifically covered by reference to a specific nucleic acid sequence.

In the case where the inserted polynucleotide sequence is transcribed and translated to produce a functional polypeptide, one of skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same polypeptide. These variants are specifically covered by the terms "nucleic acid encoding a gene product". In addition, the term specifically includes those sequences substantially identical (determined as described below) with an polynucleotide sequence disclosed here and that encode polypeptides that are either mutants of wild type polypeptides or retain the function of the polypeptide (*e.g.*, resulting from conservative substitutions of amino acids in the polypeptides). In addition, variants can be those that encode dominant negative mutants as described below.

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. When percentage of sequence identity is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions,

the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated according to, e.g., the algorithm of Meyers & Miller, *Computer Applic. Biol. Sci.* 4:11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

The phrase "substantially identical," in the context of two nucleic acids or polypeptides, refers to a sequence or subsequence that has at least 40% sequence identity with a reference sequence. Alternatively, percent identity can be any integer from 40% to 100%. More preferred embodiments include at least: 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. compared to a reference sequence using the programs described herein; preferably BLAST using standard parameters, as described below. This definition also refers to the complement of a test sequence, when the test sequence has substantial identity to a reference sequence.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP,

BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection.

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al., J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al, supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments;

or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid.

Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

(*see, e.g.*, Creighton, *Proteins* (1984)).

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below.

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, highly stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. Low stringency conditions are generally selected to be about 15-30°C below the T_m. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of

the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 time background hybridization.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cased, the nucleic acids typically hybridize under moderately stringent hybridization conditions.

In the present invention, genomic DNA or cDNA comprising nucleic acids of the invention can be identified in standard Southern blots under stringent conditions using the nucleic acid sequences disclosed here. For the purposes of this disclosure, suitable stringent conditions for such hybridizations are those which include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and at least one wash in 0.2X SSC at a temperature of at least about 50°C, usually about 55°C to about 60°C, for 20 minutes, or equivalent conditions. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

A further indication that two polynucleotides are substantially identical is if the reference sequence, amplified by a pair of oligonucleotide primers, can then be used as a probe under stringent hybridization conditions to isolate the test sequence from a cDNA or genomic library, or to identify the test sequence in, e.g., a northern or Southern blot.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a non-naturally occurring seed plant, the plant comprising: (1) a first ectopically expressed polynucleotide encoding an APETALA1 gene product at least 50% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8 or a CAULIFLOWER gene product at least 50% identical to SEQ ID NO:10 or SEQ ID NO:12; and (2) a second ectopically expressed nucleic acid molecule encoding a SEP1

gene product at least 50% identical to SEQ ID NO:28, a SEP2 gene product at least 50% identical to SEQ ID NO:30, a SEP3 gene product at least 50% identical to SEQ ID NO:32 or an AGL24 gene product at least 50% identical to SEQ ID NO:38. in some embodiments, the non-naturally occurring seed plant is characterized by early reproductive development. In some embodiments, expression of the first ectopically expressed polynucleotide is increased in a tissue of a plant compared to a wild type plant. In some embodiments, expression of the second ectopically expressed polynucleotide is increased in a tissue of a plant compared to a wild type plant. In some embodiments, expression of the first ectopically expressed polynucleotide is decreased in a tissue of a plant compared to a wild type plant. In some aspects, expression of the second ectopically expressed polynucleotide is decreased in a tissue of a plant compared to a wild type plant.

The invention provides for an endogenous first ectopically expressed polynucleotide comprising a modified gene regulatory element. Alternatively, the invention provides for an endogenous second ectopically expressed polynucleotide comprising a modified gene regulatory element. For example, the non-naturally occurring seed plant is a transgenic plant comprising a first exogenous gene regulatory element operably linked to the first ectopically expressible polynucleotide and a second exogenous gene regulatory element operably linked to the second ectopically expressible polynucleotide. In some aspects, the first polynucleotide is operably linked to the first exogenous gene regulatory element in a sense orientation. In some aspects, the first polynucleotide is operably linked to the first exogenous gene regulatory element in an antisense orientation. In some aspects, the second polynucleotide is operably linked to the second exogenous gene regulatory element in a sense orientation. In some aspects, the second polynucleotide is operably linked to the second exogenous gene regulatory element in an antisense orientation.

The invention also provides methods of modulating the timing of reproductive development in a plant, the methods comprising ectopically expressing a first polynucleotide encoding an APETALA1 gene product at least 50% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8 or a CAULIFLOWER gene product at least 50% identical to SEQ ID NO:10 or SEQ ID NO:12; and ectopically expressing a second nucleic acid molecule encoding a SEP1 gene product at least 50% identical to SEQ ID NO:28, a SEP2 gene product at least 50% identical to SEQ ID NO:30, a SEP3 gene product at least 50% identical to SEQ ID NO:32 or an AGL24 gene product at least 50% identical to SEQ ID NO:38. For example, in one aspect, the invention provides for introducing a first ectopically expressed nucleic acid molecule comprising a first polynucleotide encoding an APETALA1

gene product at least 50% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8 or a CAULIFLOWER gene product at least 50% identical to SEQ ID NO:10 or SEQ ID NO:12; and introducing a second ectopically expressed nucleic acid molecule comprising a second polynucleotide encoding a SEP1 gene product at least 50% identical to SEQ ID NO:28, a SEP2 gene product at least 50% identical to SEQ ID NO:30, a SEP3 gene product at least 50% identical to SEQ ID NO:32 or an AGL24 gene product at least 50% identical to SEQ ID NO:38.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides the surprising finding that ectopic expression of certain MADS-box-containing gene products, such as SEP1, SEP2, SEP3 or AGL24, combined with the ectopic expression of AP1, CAL or LFY gene products, result in modulated reproductive development. Thus, this invention provides plants comprising such ectopically expressible gene products as well as methods of modulating the timing of reproductive development in plants.

A flower, like a leaf or shoot, is derived from the shoot apical meristem, which is a collection of undifferentiated cells set aside during embryogenesis. The production of vegetative structures, such as leaves or shoots, and of reproductive structures, such as flowers, is temporally segregated, such that a leaf or shoot arises early in a plant life cycle, while a flower develops later. The transition from vegetative to reproductive development is the consequence of a process termed floral induction (Yanofsky, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 46:167-188 (1995), which is incorporated herein by reference).

Once induced, shoot apical meristem either persists and produces floral meristem, which gives rise to flowers, and lateral meristem, which gives rise to branches, or is itself converted to floral meristem. Floral meristem differentiates into a single flower having a fixed number of floral organs in a whorled arrangement. Dicots, for example, contain four whorls (concentric rings), in which sepals (first whorl) and petals (second whorl) surround stamens (third whorl) and carpels (fourth whorl).

Following the transition from vegetative to reproductive development in *Arabidopsis*, flower meristems arise on the flanks of the shoot apical (inflorescence) meristem and subsequently develop into flowers with four organ types (sepals, petals, stamens and carpels). Flower meristem identity is specified in part by the *APETALA1* (*AP1*), *CALIFLOWER* (*CAL*) and *LEAFY* (*LFY*) genes. In *ap1* mutants, the sepals are transformed to leaf-like organs and the petals fail to develop. In the axils of these leaf-like organs,

secondary flowers arise which repeat the same pattern as the primary ones. Although *cal* single mutants appear wild type, *ap1 cal* double mutants display a massive proliferation of inflorescence-like meristems in positions that would normally be occupied by solitary flowers. The functional redundancy shared by *AP1* and *CAL* can be explained in part by the fact that these two genes encode related members of the MADS box family of regulatory proteins (Bowman *et al.*, *Development* 119, 721-743 (1993); Gustafson-Brown *et al.*, *Cell* 76, 131-143 (1994); Kempin *et al.*, *Science* 267, 522-525 (1995); Mandel *et al.*, *Nature* 360, 273-277 (1992)).

Genetic studies led to the proposal of the ABC model that explains how the individual and combined activities of the ABC genes specify the four organ types of the typical eudicot flower. A alone specifies sepals, A and B specify petals, B and C specify stamens, and C alone specifies carpels. In *Arabidopsis*, the A-function genes are *AP1* and *APETALA2* (*AP2*), B-function genes are *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and the C-function gene is *AGAMOUS* (*AG*). In addition, recent studies have shown that a trio of closely related genes, *SEPALLATA1/2/3* (*SEP1/2/3*), are required for petal, stamen and carpel identity, and are thus necessary for the activities of the B- and C-function genes (Pelaz *et al.*, *Nature* 405, 200-203 (2000)). Remarkably, with the exception of the *AP2* gene, all of the other organ identity genes belong to the extended family of MADS-box genes, a family that is known to include more than 44 distinct sequences in *Arabidopsis* (Alvarez-Buylla *et al.*, *Proc. Natl. Acad. Sci. USA* 97, 5328-5333 (2000); Davies and Schwarz-Sommer, In *Plant Promoters and Transcription Factors (Results and Problems in Cell Differentiation 20)*, (ed. L. Nover), pp. 235-258 (1994); Purugganan *et al.*, *Genetics* 140, 345-356 (1995); Rounsley *et al.*, *Plant Cell* 7, 1259-1269 (1995)).

MADS-domain proteins, well characterized in yeast (MCM1, Ammererer, *Genes Dev.* 4, 299-312 (1990)) and mammals (SRF, Norman *et al.*, *Cell* 55, 989-1003 (1988)) form dimers that bind to DNA and form ternary complexes with many unrelated proteins (Lamb and McKnight, *Trends Biochem. Sci.* 16, 417-422 (1991); Shore and Sharrocks, *Eur. J. Chem.* 229, 1-13 (1995)). A number of studies have shown that heterodimers and ternary complexes of plant MADS-domain proteins can occur, and given the overlapping expression pattern of numerous MADS-box genes, such interactions greatly increase the regulatory complexity of MADS-box genes (Davies *et al.*, *EMBO J.* 15, 4330-4343 (1996); Egea-Cortines *et al.*, *EMBO J.* 18, 5370-5379 (1999); Fan *et al.*, *Plant J.* 11, 999-1010 (1997)). The regulatory specificity of these genes is achieved through protein-protein interactions and not through different intrinsic DNA binding specificities (Krizek and

Meyerowitz, *Proc. Natl. Acad. Sci. USA* 93, 4063-4070 (1996); Shore and Sharrocks, *Eur. J. Chem.* 229, 1-13 (1995)). MADS box proteins are composed of four different domains, designated M, I, K and C. The MADS (M) domain, is highly conserved among these proteins, and is responsible for the binding to DNA in addition to its participation in homodimer formation of some proteins. The I region also participates in homodimer formation (Krizek and Meyerowitz, *supra*; Riechmann *et al.*, *Proc. Natl. Acad. Sci. USA* 93, 4793-4798 (1996)). Adjacent to the I region is the K-domain, so named, due to its similarity to the coiled-coil domain of keratin. It is absent in the non-plant proteins, and has been implicated in protein-protein interaction (Fan *et al.*, *supra*; Krizek and Meyerowitz, *supra*; Mizukami *et al.*, *Plant Cell* 8, 831-845 (1996); Moon *et al.*, *Plant Physiol.* 120, 1193-1203 (1999); Riechmann *et al.*, *supra*). The C-terminal region has been proposed to be involved in transcriptional activation (Huang *et al.*, *Plant Mol. Biol.* 28, 549-567 (1995)), and also to play a role in the formation of ternary complexes (Egea-Cortines *et al.*, *EMBO J.* 18, 5370-5379 (1999)).

Although shoot meristem and floral meristem both consist of meristemic tissue, shoot meristem is distinguishable from the more specialized floral meristem. Shoot meristem generally is indeterminate and gives rise to an unspecified number of floral and lateral meristems. In contrast, floral meristem is determinate and gives rise to the fixed number of floral organs that comprise a flower.

By convention herein, a wild-type gene sequence is represented in upper case italic letters (for example, *APETALA1*), and a wild-type gene product is represented in upper case non-italic letters (APETALA1). Further, a mutant gene allele is represented in lower case italic letters (*ap1*), and a mutant gene product is represented in lower case non-italic letters (ap1).

Genetic studies have identified a number of genes involved in regulating flower development. These genes can be classified into different groups depending on their function. Flowering time genes, for example, are involved in floral induction and regulate the transition from vegetative to reproductive growth. In comparison, the floral meristem identity genes, which are the subject matter of the present invention as disclosed herein, encode proteins that promote the conversion of shoot meristem to floral meristem in an angiosperm. In addition, floral organ identity genes encode proteins that determine whether sepals, petals, stamens or carpels are formed during floral development (Yanofsky, *supra*, 1995; Weigel, *Ann. Rev. Genetics* 29:19-39 (1995), which is incorporated herein by

reference). Some of the floral meristem identity gene products also have a role in specifying floral organ identity.

Floral meristem identity genes have been identified by characterizing genetic mutations that prevent or alter floral meristem formation. Among floral meristem identity gene mutations in *Arabidopsis thaliana*, those in the gene *LEAFY (LFY)* generally have the strongest effect on floral meristem identity. Mutations in *LFY* completely transform the basal-most flowers into secondary shoots and have variable effects on later-arising (apical) flowers. In comparison, mutations in the floral meristem identity gene *APETALA1 (AP1)* result in replacement of a few basal flowers by inflorescence shoots that are not subtended by leaves. An apical flower produced in an *ap1* mutant has an indeterminate structure, in which a flower arises within a flower. These mutant phenotypes indicate that both *AP1* and *LFY* contribute to establishing the identity of the floral meristem although neither gene is absolutely required. The phenotype of *lfy ap1* double mutants, in which structures with flower-like characteristics are very rare, indicates that *LFY* and *AP1* encode partially redundant activities.

In addition to the *LFY* and *AP1* genes, a third locus that greatly enhances the *ap1* mutant phenotype has been identified in *Arabidopsis*. This locus, designated *CAULIFLOWER (CAL)*, derives its name from the resulting "cauliflower" phenotype, which is strikingly similar to the common garden variety of cauliflower (Kempin et al., *Science* 267:522-525 (1995), which is incorporated herein by reference). In an *ap1 cal* double mutant, floral meristem behaves as shoot meristem in that there is a massive proliferation of meristems in the position that normally would be occupied by a single flower. However, an *Arabidopsis* mutant lacking only CAL, such as *cal-1*, has a normal phenotype, indicating that AP1 can substitute for the loss of CAL in these plants. In addition, because floral meristem that forms in an *ap1* mutant behaves as shoot meristem in an *ap1 cal* double mutant, CAL can largely substitute for AP1 in specifying floral meristem. These genetic data indicate that *CAL* and *AP1* encode activities that are partially redundant in converting shoot meristem to floral meristem.

Other genetic loci play at least minor roles in specifying floral meristem identity. For example, although a mutation in *APETALA2 (AP2)* alone does not result in altered inflorescence characteristics, *ap2 ap1* double mutants have indeterminate flowers (flowers with shoot-like characteristics; Bowman et al., *Development* 119:721-743 (1993), which is incorporated herein by reference). Also, mutations in the *CLAVATA1 (CLV1)* gene result in an enlarged meristem and lead to a variety of phenotypes (Clark et al., *Development*

119:397-418 (1993)). In a *clv1 ap1* double mutant, formation of flowers is initiated, but the center of each flower often develops as an indeterminate inflorescence. Thus, mutations in *CLAVATA1* result in the loss of floral meristem identity in the center of wild-type flowers. Genetic evidence also indicates that the gene product of *UNUSUAL FLORAL ORGANS (UFO)* plays a role in determining the identity of floral meristem. Additional floral meristem identity genes associated with altered floral meristem formation remain to be isolated.

Mutations in another locus, designated *TERMINAL FLOWER (TFL)*, produce phenotypes that generally are reversed as compared to mutations in the floral meristem identity genes. For example, *tfl* mutants flower early, and the indeterminate apical and lateral meristems develop as determinate floral meristems (Alvarez et al., *Plant J.* 2:103-116 (1992)). These characteristics indicate that the TFL promotes maintenance of shoot meristem. *TFL* also acts directly or indirectly to negatively regulate AP1 and LFY expression in shoot meristem since these AP1 and LFY are ectopically expressed in the shoot meristem of *tfl* mutants (Gustafson-Brown et al., *Cell* 76:131-143 (1994); Weigel et al., *Cell* 69:843-859 (1992)). It is recognized that a plant having a mutation in TFL can have a phenotype similar to a non-naturally occurring seed plant of the invention. Such *tfl* mutants, however, which have a mutation in an endogenous *TERMINAL FLOWER* gene, are explicitly excluded from the scope of the present invention.

The results of such genetic studies indicate that several floral meristem identity gene products, including AP1, CAL and LFY, act redundantly to convert shoot meristem to floral meristem in an angiosperm. As disclosed herein, ectopic expression of a single floral meristem identity gene product such as AP1, CAL or LFY is sufficient to convert shoot meristem to floral meristem in an angiosperm. Thus, the present invention provides a non-naturally occurring seed plant such as an angiosperm or gymnosperm that contains a first or second ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product, provided that such ectopic expression is not due to a mutation in an endogenous *TERMINAL FLOWER* gene.

As disclosed herein, an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be, for example, a transgene encoding a floral meristem identity gene product under control of a heterologous gene regulatory element. In addition, such an ectopically expressible nucleic acid molecule can be an endogenous floral meristem identity gene coding sequence that is placed under control of a heterologous gene regulatory element. The ectopically expressible nucleic acid molecule also can be, for example, an endogenous floral meristem identity gene having a modified gene regulatory

element such that the endogenous floral meristem identity gene is no longer subject to negative regulation by TFL.

The term "ectopically expressible" is used herein to refer to a nucleic acid molecule encoding a floral meristem identity gene product that can be expressed in a tissue other than a tissue in which it normally is expressed or at a time other than the time at which it normally is expressed, provided that the floral meristem identity gene product is not expressed from its native, naturally occurring promoter. Ectopic expression of a floral meristem identity gene product is a result of the expression of the gene coding region from a heterologous promoter or from a modified variant of its own promoter, such that expression of the floral meristem identity gene product is no longer in the tissue in which it normally is expressed or at the time at which it normally is expressed. An exogenous nucleic acid molecule encoding an AP1 gene product under control of its native, wild type promoter, for example, does not constitute an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product. However, a nucleic acid molecule encoding an AP1 gene product under control of a constitutive promoter, which results in expression of AP1 in a tissue such as shoot meristem where it is not normally expressed, is an ectopically expressible nucleic acid molecule as defined herein.

Actual ectopic expression of a floral meristem identity gene is dependent on various factors and can be constitutive or inducible expression. For example, AP1, which normally is expressed in floral meristem, is ectopically expressible in the shoot meristem of an angiosperm. When a floral meristem identity gene product such as AP1, CAL or LFY is ectopically expressed in shoot meristem in an angiosperm, the shoot meristem is converted to floral meristem and early reproductive development can occur (*see* WO 97/46078, incorporated herein by reference).

An ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be expressed prior to the time in development at which the corresponding endogenous gene normally is expressed. For example, an *Arabidopsis* plant grown under continuous light conditions expresses *AP1* just prior to day 18, when normal reproductive development (flowering) begins. However, *AP1* can be ectopically expressed in shoot meristem prior to day 18, resulting in early conversion of shoot meristem to floral meristem and early reproductive development. *See* WO 97/46078. As disclosed in Example ID of WO 97/46078, a transgenic *Arabidopsis* plant that ectopically expresses *AP1* in shoot meristem under control of a constitutive promoter can flower at day 10, which is earlier than the time of reproductive development for a non-transgenic plant grown under the same

conditions (day 18). It is recognized that in some transgenic seed plants containing, for example, an exogenous nucleic acid molecule encoding AP1 under control of a constitutive promoter, neither the exogenous nor endogenous AP1 will be expressed. Such transgenic plants in which AP1 gene expression is cosuppressed, although not characterized by early reproductive development, also can be valuable as disclosed below.

I. Floral Meristem Gene Products

As used herein, the term "floral meristem identity gene product" means a gene product that promotes conversion of shoot meristem to floral meristem in an angiosperm. Expression of a floral meristem identity gene product such as AP1, CAL or LFY in shoot meristem can convert shoot meristem to floral meristem in an angiosperm. Furthermore, ectopic expression of a floral meristem identity gene product also can promote early reproductive development.

A floral meristem identity gene product is distinguishable from a late flowering gene product or an early flowering gene product. The use of a late flowering gene product or an early flowering gene product is not encompassed within the scope of the present invention. In addition, reference is made herein to an "inactive" floral meristem identity gene product, as exemplified by the product of the *Brassica oleracea* var. *botrytis CAL* gene *(BobCAL)* (see below). Expression of an inactive floral meristem identity gene product in an angiosperm does not result in the conversion of shoot meristem to floral meristem in the angiosperm. An inactive floral meristem identity gene product such as BobCAL is excluded from the meaning of the term "floral meristem identity gene product" as defined herein.

A. AP1

A floral meristem identity gene product can be, for example, an AP1 gene product having the amino acid sequence of SEQ ID NO: 2, which is a 256 amino acid gene product encoded by the *Arabidopsis thaliana AP1* cDNA. The *Arabidopsis AP1* cDNA encodes a highly conserved MADS domain, which can function as a DNA-binding domain, and a K domain, which has structural similarity to the coiled-coil domain of keratins and can be involved in protein-protein interactions.

As used herein, the term "APETALA1," "AP1" or "AP1 gene product" means a floral meristem identity gene product that is characterized, in part, by having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2 in the region from amino acid 1 to amino acid 163 or with the amino acid sequence of SEQ ID NO: 8 in the region from amino acid 1 to amino acid 163. Alternatively, "AP1 gene product" refers to a gene product substantially identical to SEQ ID NO:2 or SEQ ID NO:8. Like other floral meristem identity gene products, AP1 promotes conversion of shoot meristem to floral meristem in an angiosperm. An AP1 gene product useful in the invention can be, for example, *Arabidopsis* AP1 having the amino acid sequence of SEQ ID NO: 2; *Brassica oleracea* AP1 having the amino acid sequence of SEQ ID NO: 4; *Brassica oleracea* var. *botrytis* AP1 having the amino acid sequence of SEQ ID NO: 6 or *Zea mays* AP1 having the amino acid sequence of SEQ ID NO: 8.

In wild-type *Arabidopsis*, *AP1* RNA is expressed in flowers but is not detectable in roots, stems or leaves (Mandel et al., *Nature* 360:273-277 (1992), which is incorporated herein by reference). The earliest detectable expression of *AP1* RNA is in young floral meristem at the time it initially forms on the flanks of shoot meristem. Expression of *AP1* increases as the floral meristem increases in size; no *AP1* expression is detectable in shoot meristem. In later stages of development, *AP1* expression ceases in cells that will give rise to reproductive organs of a flower (stamens and carpels), but is maintained in cells that will give rise to non-reproductive organs (sepals and petals; Mandel, *supra*, 1992). Thus, in nature, *AP1* expression is restricted to floral meristem and to certain regions of the flowers that develop from this meristemic tissue.

B. CAL

CAULIFLOWER (CAL) is another example of a floral meristem identity gene product. As used herein, the term "CAULIFLOWER," "CAL" or "CAL gene product" means a floral meristem identity gene product that is characterized, in part, bysubstantial identity to an amino acid sequence of SEQ ID NO: 10 in the region from amino acid 1 to amino acid 160 or with the amino acid sequence of SEQ ID NO: 12 in the region from amino acid 1 to amino acid 160. Alternatively, "CAL gene product" refers to a gene product substantially identical to SEQ ID NO:10 or SEQ ID NO:12.

A CAL gene product is exemplified by the *Arabidopsis* CAL gene product, which has the amino acid sequence of SEQ ID NO: 10, or the *Brassica oleracea* CAL gene

product, which has the amino acid sequence of SEQ ID NO:12. As disclosed herein, CAL, like AP1, contains a MADS domain and a K domain. The MADS domains of CAL and AP1 differ in only five of 56 amino acid residues, where four of the five differences represent conservative amino acid replacements. Over the entire sequence, the *Arabidopsis* CAL and *Arabidopsis* AP1 sequences (SEQ ID NOS: 10 and 2) are 76% identical and are 88% similar if conservative amino acid substitutions are allowed.

Similar to the expression pattern of *AP1*, *CAL* RNA is expressed in young floral meristem in *Arabidopsis*. However, in contrast to *AP1* expression, which is high throughout sepal and petal development, *CAL* expression is low in these organs. Thus, in nature, *CAL* is expressed in floral meristem and, to a lesser extent, in the organs of developed flowers.

The skilled artisan will recognize that, due to the high sequence conservation between AP1 and CAL, a novel ortholog can be categorized as both a CAL and an AP1, as defined herein. However, if desired, an AP1 ortholog can be distinguished from a CAL ortholog by demonstrating a greater similarity to *Arabidopsis* AP1 than to any other MADS box gene, such as CAL, as set forth in Purugganan et al. (*Genetics* 140:345-356 (1995), which is incorporated herein by reference). Furthermore, AP1 can be distinguished from CAL by its ability to complement, or restore a wild-type phenotype, when introduced into a strong *ap1* mutant. For example, introduction of *Arabidopsis AP1 (AGL7)* complements the phenotype of the strong *ap1-1* mutant; however, introduction of *CAL (AGL10)* into a *cal-1 ap1-1* mutant plant yields the *ap1-1* single mutant phenotype, demonstrating that *CAL* cannot complement the *ap1-1* mutation (see, for example, Mandel et al., *supra,* 1992; Kempin et al., *supra,* 1995). Thus, AP1 can be distinguished from CAL, if desired, by the ability of a nucleic acid molecule encoding AP1 to complement a strong *ap1* mutant such as *ap1-1* or *ap1-15*.

C. LFY

LEAFY (LFY) is yet another example of a floral meristem identity gene product. As used herein, the term "LEAFY" or "LFY" or "LFY gene product" means a floral meristem identity gene product that is characterized, in part, by having an amino acid sequence that has substantial identity with the amino acid sequence of SEQ ID NO:16. In nature, LFY is expressed in floral meristem as well as during vegetative development. As disclosed herein, ectopic expression in shoot meristem of a floral meristem identity gene

product, which normally is expressed in floral meristem, can convert shoot meristem to floral meristem in an angiosperm. Under appropriate conditions, ectopic expression in shoot meristem of a floral meristem identity gene product such as AP1, CAL, LFY, or a combination thereof, can promote early reproductive development.

D. Floral Meristem Gene Product Orthologs

Flower development in *Arabidopsis* is recognized in the art as a model for flower development in angiosperms in general. Gene orthologs corresponding to the *Arabidopsis* genes involved in the early steps of flower formation have been identified in distantly related angiosperm species, and these gene orthologs show remarkably similar patterns of RNA expression. Mutations in gene orthologs also result in phenotypes that correspond to the phenotype produced by a similar mutation in *Arabidopsis*. For example, orthologs of the *Arabidopsis* floral meristem identity genes *AP1* and *LFY* and the *Arabidopsis* organ identity genes *AGAMOUS*, *APETALA3* and *PISTILLATA* have been isolated from monocots such as maize and, where characterized, reveal the anticipated RNA expression patterns and related mutant phenotypes (Schmidt et al., *Plant Cell* 5:729-737 (1993); and Veit et al., *Plant Cell* 5:1205-1215 (1993), each of which is incorporated herein by reference). Furthermore, a gene ortholog can be functionally interchangeable in that it can function across distantly related species boundaries (Mandel et al., *Cell* 71:133-143 (1992), which is incorporated herein by reference). Taken together, these data suggest that the underlying mechanisms controlling the initiation and proper development of flowers are conserved across distantly related dicot and monocot boundaries.

Floral meristem identity genes in particular are conserved among distantly related angiosperm and gymnosperm species. For example, a gene ortholog of *Arabidopsis AP1* has been isolated from *Antirrhinum majus* (snapdragon; Huijser et al., *EMBO J.* 11:1239-1249 (1992), which is incorporated herein by reference). An ortholog of *Arabidopsis AP1* also has been isolated from *Brassica oleracea* var. *botrytis* (cauliflower, *see* SEQ ID NO:6), *Zea Mays* (maize; *see* SEQ ID NO:8) and rice (OsMADS14 (*Plant Physiology* 120:1193-1203 (1999)). Furthermore, *AP1* orthlogs also can be isolated from gymnosperms. Similarly, gene orthologs of *Arabidopsis LFY* have been isolated from angiosperms such as *Antirrhinum majus*, tobacco and poplar tree and from gymnosperms such as Douglas fir (Coen et al., *Cell*, 63:1311-1322 (1990); Kelly et al., *Plant Cell* 7:225-234 (1995); and Rottmann et al., *Cell Biochem. Suppl.* 17B: 23 (1993); Strauss et al.,

Molec. Breed 1:5-26 (1995), each of which is incorporated herein by reference). The conservation of floral meristem identity gene products in non-flowering plants such as coniferous trees indicates that floral meristem identity genes can promote the reproductive development of gymnosperms as well as angiosperms.

The characterization of *ap1* and *lfy* mutants also indicates that floral meristem identity gene products such as AP1 and LFY function similarly in distantly related plant species. For example, a mutation in the *Antirrhinum AP1* ortholog results in a phenotype similar to the *Arabidopsis ap1* indeterminate flower within a flower phenotype (Huijser et al., *supra*, 1992). In addition, a mutation in the *Antirrhinum LFY* ortholog results in a phenotype similar to the *Arabidopsis lfy* mutant phenotype (Coen et al., *supra*, 1995)

A floral meristem identity gene product also can function across species boundaries. For example, introduction of a nucleic acid molecule encoding *Arabidopsis* LFY into a heterologous seed plant such as tobacco or aspen results in early reproductive development (Weigel and Nilsson, *Nature* 377:495-500 (1995), which is incorporated herein by reference). As disclosed herein, a nucleic acid molecule encoding an *Arabidopsis* AP1 gene product (SEQ ID NO: 1) or an *Arabidopsis* CAL gene product (SEQ ID NO: 9) can be introduced into a heterologous seed plant such as corn, wheat, rice or pine and, upon ectopic expression, can promote early reproductive development in the transgenic seed plant. Furthermore, as disclosed herein, the conserved nature of the *AP1*, *CAL* and *LFY* coding sequences among diverse seed plant species allows a nucleic acid molecule encoding a floral meristem identity gene product isolated from essentially any seed plant to be introduced into essentially any other seed plant, wherein, upon appropriate expression of the introduced nucleic acid molecule in the seed plant, the floral meristem identity gene product promotes early reproductive development in the seed plant.

If desired, a novel *AP1, CAL* or *LFY* coding sequence can be isolated from a seed plant using a nucleotide sequence as a probe and methods well known in the art of molecular biology (Sambrook et al. (eds.), *Molecular Cloning: A Laboratory Manual* (Second Edition), Plainview, NY: Cold Spring Harbor Laboratory Press (1989), which is incorporated herein by reference). As exemplified herein and discussed in detail below (see Example VA), an *AP1* ortholog from *Zea Mays* (maize; SEQ ID NO: 7) was isolated using the *Arabidopsis AP1* cDNA (SEQ ID NO: 1) as a probe.

II. AGAMOUS-like Gene Products

Modulation of expression of the gene products described below, either alone, or in combination with the ectopic expression of AP1 or CAL, results in the modulation of the development of reproductive development in plants.

A. SEP1, SEP2 and SEP3

SEP1, SEP2 and SEP3 (previously known as AGL2, AGL4 and AGL9, respectively) are a class of floral organ identity gene products that are required for development of stamens and carpels (Pelaz, *et al.*, *Nature* 405:200-203 (2000)). The SEP gene products are functionally redundant. Therefore, inactivation of only one SEP gene product does not typically result in the development of a mutant floral phenotype. Ectopic or increased expression of a SEP gene product results in early development of reproductive structures. Delay of reproductive development typically requires the reduction of expression of at least two and sometimes all three SEP gene products due to the redundancy of their function.

As used herein, the term "SEP1" or "SEP1 gene product" means a floral meristem identity gene product, or active fragment thereof, that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 28. The term "SEP2" or "SEP2 gene product" means a floral meristem identity gene product, or active fragment thereof, that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 30. SEP1 and SEP2 sequences were previously described in Ma *et al.*, *Genes & Development* 5:484-495 (1991). An exemplary SEP1 nucleic acid sequence is displayed as SEQ ID NO:27. An exemplary SEP2 nucleic acid sequence is displayed as SEQ ID NO:29. The term "SEP3" or "SEP3 gene product" means a floral meristem identity gene product, or active fragment thereof, that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 32. SEP3 sequences were previously described in Mandel *et al.*, *Sex. Plant Reprod.* 11:22-28 (1998). An exemplary SEP3 nucleic acid sequence is displayed as SEQ ID NO:31.

B. AGL20

As used herein, the term "AGL20" or "AGL20 gene product" means a gene product that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 34. AGL20 is also known as "SOC1." *See, e.g.*, Samach *et al.* *Science* 288:1613-1616 (2000). Reduction of endogenous expression of AGL20 results in delayed development of reproductive structures in plants. An exemplary AGL20 nucleic acid sequence is displayed as SEQ ID NO:33.

C. AGL22

As used herein, the term "AGL22" or "AGL22 gene product" means a gene product that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 36. Decreased expression of an AGL22 gene product results in early development of reproductive structures. AGL22 is also known as "SVP." An exemplary AGL22 nucleic acid sequence is displayed as SEQ ID NO:35.

D. AGL24

As used herein, the term "AGL24" or "AGL24 gene product" means a gene product that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 38. An exemplary AGL24 nucleic acid sequence is displayed as SEQ ID NO:37. Ectopic or increased expression of AGL24 results in early development of reproductive structures in plants. Reduced expression of endogenous AGL24 results in delayed development of reproductive structures in plants.

E. AGL27

As used herein, the term "AGL27" or "AGL27 gene product" means a gene product that is characterized, in part, by having an amino acid sequence substantially identical to SEQ ID NO: 36. An exemplary AGL27 cDNA nucleic acid sequence is displayed as SEQ ID NO:39. An alternatively spliced AGL27 cDNA, and resulting translated product, are displayed as SEQ ID NO:48 and SEQ ID NO:49.

III. Effect of Gene Products of the Invention on Timing of Reproductive Development

As described in U.S. Patent 6,002,069, ectopic expression of the AP1 or CAL gene products results in the early development of reproductive structures in plants. The present invention demonstrates that ectopic expression of a number of other genes in combination with the ectopic expression of AP1 or CAL, leads to significantly earlier timing of reproductive development than plants ectopically expressing AP1 or CAL alone. In one embodiment, the invention provides a non-naturally occurring seed plant that contains a first ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product, provided that the first nucleic acid molecule is not ectopically expressed due to a

mutation in an endogenous *TERMINAL FLOWER* gene, and a second ectopically expressible nucleic acid molecule encoding SEP1, SEP2, SEP3 or AGL24, wherein the plant is characterized by modulated timing of reproductive development.

As used herein, the term "characterized by early reproductive development" when used in reference to a non-naturally occurring seed plant of the invention, means a non-naturally occurring seed plant that forms reproductive structures at an earlier stage than when reproductive structures form on a corresponding naturally occurring seed plant that is grown under the same conditions and that does not ectopically express a floral meristem identity gene product. In addition, "characterized by early reproductive development" also refers to the formation of reproduction structures at an earlier stage than a plant identical except for the lack of ectopic expression of the nucleic acids of the invention (e.g., polynucleotides substantially similar to nucleic acid molecules encoding SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 or SEQ ID NO:40). Note that "stage," as used above, refers to either the amount of time from germination of seed or the number of leaves that a plant produces prior to initiation of reproductive structures. Similarly, "characterized by late reproductive development" or "characterized by delayed reproductive development" refers to the delayed development of reproductive structures compared to a naturally-occurring seed plant or to a plant, natural or transgenic, that does not ectopically express a nucleic acid of the invention. The reproductive structure of an angiosperm, for example, is a flower, and the reproductive structure of a coniferous plant is a cone. For a particular naturally occurring seed plant, reproductive development occurs at a well-defined time that depends, in part, on genetic factors as well as on environmental conditions, such as day length and temperature. Thus, given a defined set of environmental condition and lacking ectopic expression of a floral meristem identity gene product, a naturally occurring seed plant will undergo reproductive development at a relatively fixed time.

It is recognized that various transgenic plants that are characterized by altered timing of reproductive development have been described previously. Such transgenic plants, as discussed herein, are distinguishable from a non-naturally occurring seed plant of the invention or are explicitly excluded from the present invention. The product of a "late-flowering gene" can promote early reproductive development. However, a late flowering gene product is not a floral meristem identity gene product since it does not specify the conversion of shoot meristem to floral meristem in an angiosperm. Therefore, a

transgenic plant expressing a late-flowering gene product is distinguishable from a non-naturally occurring seed plant of the invention. For example, a transgenic plant expressing the late-flowering gene, *CONSTANS (CO)*, flowers earlier than the corresponding wild type plant, but does not contain an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product (Putterill et al., *Cell* 80:847-857 (1995)). Thus, the early-flowering transgenic plant described by Putterill et al. is not a non-naturally occurring seed plant as defined herein.

Early reproductive development also has been observed in a transgenic tobacco plant expressing an exogenous rice MADS domain gene. Although the product of the rice MADS domain gene promotes early reproductive development, it does not specify the identity of floral meristem and, thus, cannot convert shoot meristem to floral meristem in an angiosperm (Chung et al., *Plant Mol. Biol.* 26:657-665 (1994)). Therefore, an early-flowering transgenic plant containing this rice MADS domain gene, like an early-flowering transgenic plant containing *CONSTANS*, is distinguishable from an early-flowering non-naturally occurring seed plant of the invention.

Mutations in a class of genes known as "early-flowering genes" also produce plants characterized by early reproductive development. Such early-flowering genes include, for example, *EARLY FLOWERING 1-3 (ELF1, ELF2, ELF3); EMBRYONIC FLOWER 1,2 (EMF1, EMF2); LONG HYPOCOTYL 1,2 (HY1, HY2); PHYTOCHROME B (PHYB), SPINDLY (SPY)* and *TERMINAL FLOWER (TFL)* (Weigel, *supra*, 1995). The wild type product of an early-flowering gene retards reproductive development and is distinguishable from a floral meristem identity gene product in that an early-flowering gene product does not promote conversion of shoot meristem to floral meristem in an angiosperm. A plant that flowers early due to the loss of an early-flowering gene product function is distinct from a non-naturally occurring seed plant of the invention characterized by early reproductive development since such a plant does not contain an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product.

An *Arabidopsis* plant having a mutation in the *TERMINAL FLOWER (TFL)* gene is characterized by early reproductive development and by the conversion of shoots to flowers (Alvarez et al., *Plant J.* 2:103-116 (1992), which is incorporated herein by reference). However, TFL is not a floral meristem identity gene product, as defined herein. Specifically, it is the loss of TFL that promotes conversion of shoot meristem to floral meristem. Since the function of TFL is to antagonize formation of floral meristem, a *tfl* mutant, which lacks functional TFL, converts shoot meristem to floral meristem prematurely. Although TFL is

not a floral meristem identity gene product and does not itself convert shoot meristem to floral meristem, the loss of TFL can result in a plant with an ectopically expressed floral meristem identity gene product. However, such a *tfl* mutant, in which a mutation in an endogenous *TERMINAL FLOWER* gene results in conversion of shoot meristem to floral meristem, is excluded explicitly from the present invention.

In various embodiments, the present invention provides a non-naturally occurring seed plant containing a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product, provided that the first nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene. If desired, a non-naturally occurring seed plant of the invention can contain a second ectopically expressible nucleic acid molecule encoding SEP1, SE2, SEP3, AGL20, AGL22, AGL24, or AGL27, provided that the first or second nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene.

An ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be expressed, as desired, either constitutively or inducibly. Such an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be an endogenous floral meristem identity gene that has, for example, a mutation in a gene regulatory element. An ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product also can be an endogenous nucleic acid molecule encoding a floral meristem identity gene product that is linked to an exogenous, heterologous gene regulatory element that confers ectopic expression. In addition, an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product can be an exogenous nucleic acid molecule that encodes a floral meristem identity gene product under control of a heterologous gene regulatory element.

A non-naturally occurring seed plant of the invention can contain an endogenous floral meristem identity gene having a modified gene regulatory element. The term "modified gene regulatory element," as used herein in reference to the regulatory element of a floral meristem identity gene, means a regulatory element having a mutation that results in ectopic expression of the linked endogenous floral meristem identity gene. Such a gene regulatory element can be, for example, a promoter or enhancer element and can be positioned 5' or 3' to the coding sequence or within an intronic sequence of the floral meristem identity gene. A modified gene regulatory element can have, for example, a nucleotide insertion, deletion or substitution that is produced, for example, by chemical mutagenesis using a mutagen such as ethylmethane sulfonate or by insertional mutagenesis

using a transposable element. A modified gene regulatory element can be a functionally inactivated binding site for TFL or a functionally inactivated binding site for a gene product regulated by TFL, such that modification of the gene regulatory element results in ectopic expression of the linked floral meristem identity gene product, for example, in the shoot meristem of an angiosperm.

The present invention also provides a transgenic seed plant containing a first exogenous gene promoter that regulates a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second exogenous gene promoter that regulates a second ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product.

The present invention further provides a transgenic seed plant containing a first exogenous ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second exogenous gene promoter that regulates a second ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product, provided that the first nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene.

The invention also provides, therefore, a plant characterized by modulated (delayed or early) reproductive development, the plant containing a sense or antisense nucleic acid molecule encoding AP1, or a fragment thereof; a sense or antisense nucleic acid molecule encoding CAL, or a fragment thereof; and a sense or antisense nucleic acid molecule encoding LFY, or a fragment thereof, such that expression of AP1 and LFY gene products, including expression of endogenous AP1 and LFY gene products, is suppressed in the transgenic seed plant. Similarly, a sense or antisense nucleic acid molecule encoding SEP1, or a fragment thereof, a sense or antisense nucleic acid molecule encoding SEP2, or a fragment thereof, a sense or antisense nucleic acid molecule encoding SEP3, or a fragment thereof, a sense or antisense nucleic acid molecule encoding AGL20, or a fragment thereof, a sense or antisense nucleic acid molecule encoding AGL22, or a fragment thereof, a sense or antisense nucleic acid molecule encoding AGL24, or a fragment thereof, a sense or antisense nucleic acid molecule encoding AGL27, or a fragment thereof can also be used singly, in combination with each other or in combination with any of the AP1, CAL or LFY constructs discussed above.

In addition, the invention provides a transgenic seed plant containing a first exogenous ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product, provided that the first second nucleic acid molecule is not ectopically

expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene, and further containing a second exogenous ectopically expressible nucleic acid molecule encoding a second floral meristem identity gene product, where the first floral meristem identity gene product is different from the second floral meristem identity gene product.

As disclosed herein, ectopic expression of two different floral meristem identity gene products can be particularly useful. For example, a fraction of the progeny of a cross between a transgenic *Arabidopsis* line constitutively expressing *AP1* under control of the cauliflower mosaic virus 35S promoter and a transgenic *Arabidopsis* line constitutively expressing *LFY* under control of the cauliflower mosaic virus 35S promoter are characterized by enhanced early reproductive development as compared to the early reproductive development of 35S-*AP1* transgenic lines or 35S-*LFY* transgenic lines. These results indicate that ectopic expression of the combination of AP1 and LFY in a seed plant can result in enhanced early reproductive development as compared to the early reproductive development obtained by ectopic expression of AP1 or LFY alone. Similarly, the ectopic expression of the combination of at least one of AP1 and CALIFLOWER with at least one of SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 results in early reproductive development. Thus, by using a combination of two different floral meristem identity gene products, plant breeding, for example, can be accelerated further as compared to the use of a single floral meristem identity gene product.

A useful combination of first and second floral meristem identity gene products can be, for example, AP1 and SEP3, CAL and SEP3, AP1 and AGL24 or CAL and AGL24. Where a transgenic seed plant of the invention contains first and second exogenous nucleic acid molecules encoding different floral meristem identity gene products, it will be recognized that the order of introducing the first and second nucleic acid molecules into the seed plant is not important for purposes of the present invention. Thus, a transgenic seed plant of the invention having, for example, AP1 as a first floral meristem identity gene product and SEP3 as a second floral meristem identity gene product is equivalent to a transgenic seed plant having SEP3 as a first floral meristem identity gene product and AP1 as a second floral meristem identity gene product.

IV. Plant Transformation

As used herein, the term "introducing," when used in reference to a nucleic acid molecule and a seed plant such as an angiosperm or a gymnosperm, means transferring

an exogenous nucleic acid molecule into the seed plant. For example, an exogenous nucleic acid molecule encoding a floral meristem identity gene product can be introduced into a seed plant by a variety of methods including *Agrobacterium*-mediated transformation or direct gene transfer methods such as electroporation or microprojectile-mediated transformation.

Transformation methods based upon the soil bacterium *Agrobacterium tumefaciens,* known as "agro-infection," are useful for introducing a nucleic acid molecule into a broad range of angiosperms and gymnosperms. The wild type form of *Agrobacterium* contains a Ti (tumor-inducing) plasmid that directs production of tumorigenic crown gall growth on host plants. Transfer of the tumor-inducing T-DNA region of the Ti plasmid to a plant genome requires the Ti plasmid-encoded virulence genes as well as T-DNA borders, which are a set of direct DNA repeats that delineate the region to be transferred. *Agrobacterium*-based vector is a modified form of a Ti plasmid, in which the tumor inducing functions are replaced by nucleic acid sequence of interest to be introduced into the plant host.

Current protocols for *Agrobacterium*-mediated transformation employ cointegrate vectors or, preferably, binary vector systems in which the components of the Ti plasmid are divided between a helper vector, which resides permanently in the *Agrobacterium* host and carries the virulence genes, and a shuttle vector, which contains the gene of interest bounded by T-DNA sequences. A variety of binary vectors are well known in the art and are commercially available from, for example, Clontech (Palo Alto, California). Methods of coculturing *Agrobacterium* with cultured plant cells or wounded tissue such as leaf tissue, root explants, hypocotyledons, stem pieces or tubers, for example, also are well known in the art (Glick and Thompson (eds.), Methods in Plant Molecular Biology and Biotechnology, Boca Raton, FL: CRC Press (1993), which is incorporated herein by reference). Wounded cells within the plant tissue that have been infected by *Agrobacterium* can develop organs *de novo* when cultured under the appropriate conditions; the resulting transgenic shoots eventually give rise to transgenic plants containing the exogenous nucleic acid molecule of interest, as described in Example I.

Agrobacterium-mediated transformation has been used to produce a variety of transgenic seed plants (see, for example, Wang et al. (eds), Transformation of Plants and Soil Microorganisms, Cambridge, UK: University Press (1995), which is incorporated herein by reference). For example, *Agrobacterium*-mediated transformation can be used to produce transgenic crudiferous plants such as *Arabidopsis*, mustard, rapeseed and flax; transgenic leguminous plants such as alfalfa, pea, soybean, trefoil and white clover; and transgenic

solanaceous plants such as eggplant, petunia, potato, tobacco and tomato. In addition, *Agrobacterium*-mediated transformation can be used to introduce exogenous nucleic acids into apple, aspen, belladonna, black currant, carrot, celery, cotton, cucumber, grape, horseradish, lettuce, morning glory, muskmelon, neem, poplar, strawberry, sugar beet, sunflower, walnut and asparagus plants (see, for example, Glick and Thompson, *supra,* 1993).

Microprojectile-mediated transformation also is a well known method of introducing an exogenous nucleic acid molecule into a variety of seed plant species. This method, first described by Klein et al., *Nature* 327:70-73 (1987), which is incorporated herein by reference, relies on microprojectiles such as gold or tungsten that are coated with the desired nucleic acid molecule by precipitation with calcium chloride, spermidine or PEG. The microprojectile particles are accelerated at high speed into seed plant tissue using a device such as the Biolistic™ PD-1000 (Biorad, Hercules, California).

Microprojectile-mediated delivery or "particle bombardment" is especially useful to transform seed plants that are difficult to transform or regenerate using other methods. Microprojectile-mediated transformation has been used, for example, to generate a variety of transgenic seed plant species, including cotton, tobacco, corn, hybrid poplar and papaya (see, for example, Glick and Thompson, *supra,* 1993). The transformation of important cereal crops such as wheat, oat, barley, sorghum and rice also has been achieved using microprojectile-mediated delivery (Duan et al., *Nature Biotech.* 14:494-498 (1996); Shimamoto, *Curr. Opin. Biotech.* 5:158-162 (1994), each of which is incorporated herein by reference). A rapid transformation regeneration system for the production of transgenic plants, such as transgenic wheat, in two to three months also can be useful in producing a transgenic seed plant of the invention (European Patent No. EP 0 709 462 A2, Application number 95870117.9, filed 25 October 1995, which is incorporated herein by reference).

Thus, a variety of methods for introducing a nucleic acid molecule into a seed plant are well known in the art. Important crop species such as rice, for example, have been transformed using microprojectile delivery, *Agrobacterium*-mediated transformation or protoplast transformation (Hiei et al., *The Plant J.* 6(2):271-282 (1994); Shimamoto, *Science* 270:1772-1773 (1995), each of which is incorporated herein by reference). Fertile transgenic maize has been obtained, for example, by microparticle bombardment (see Wang et al., *supra,* 1995). As discussed above, barley, wheat, oat and other small-grain cereal crops also have been transformed, for example, using microparticle bombardment (see Wang et al., *supra,* 1995).

Methods of transforming forest trees including both angiosperms and gymnosperms also are well known in the art. Transgenic angiosperms such as members of the genus *Populus*, which includes aspens and poplars, have been generated using *Agrobacterium*-mediated transformation, for example. In addition, transgenic *Populus* and sweetgum, which are of interest for biomass production for fuel, also have been produced. Transgenic gymnosperms, including conifers such as white spruce and larch, also have been obtained, for example, using microprojectile bombardment (Wang et al., *supra,* 1995). The skilled artisan will recognize that *Agrobacterium*-mediated or microprojectile-mediated transformation, as disclosed herein, or other methods known in the art can be used to introduce a nucleic acid molecule encoding a floral meristem identity gene product into a seed plant according to the methods of the invention.

The nucleic acids of the invention can be used to confer desired traits on essentially any plant. Thus, the invention has use over a broad range of plants, including species from the genera *Anacardium, Arachis, Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Carthamus, Cocos, Coffea, Cucumis, Cucurbita, Daucus, Elaeis, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium, Lupinus, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannesetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna,* and *Zea*.

V. Converting Shoot Meristem to Floral Meristem

The term "converting shoot meristem to floral meristem," as used herein, means promoting the formation of flower progenitor tissue where shoot progenitor tissue otherwise would be formed in the angiosperm. As a result of the conversion of shoot meristem to floral meristem, flowers form in an angiosperm where shoots normally would form. The conversion of shoot meristem to floral meristem can be identified using well known methods, such as scanning electron microscopy, light microscopy or visual inspection (see, for example, Mandel and Yanofsky, *Plant Cell* 7:1763-1771 (1995), which is incorporated herein by reference or Weigel and Nilsson, *supra*, 1995).

Provided herein are methods of converting shoot meristem to floral meristem in an angiosperm by introducing a first ectopically expressible nucleic acid molecule encoding a first floral meristem identity gene product and a second ectopically expressible

nucleic acid molecule encoding a second floral meristem identity gene product into the angiosperm, where the first floral meristem identity gene product is different from the second floral meristem identity gene product. As discussed above, first and second floral meristem identity gene products useful in converting shoot meristem to floral meristem in an angiosperm can be, for example, AP1 and LFY, CAL and LFY, or AP1 and CAL. In other preferred embodiments, the ectopic expression of the combination of at least one of AP1 and CALIFLOWER with at least one of SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 results in conversion of shoot meristem to floral meristem.

VI. Methods of Modulating Reproductive Development

As discussed above, the present invention provides methods of promoting modulated timing of reproductive development in a seed plant by ectopically expressing a first nucleic acid molecule encoding a first floral meristem identity gene product in the seed plant, provided that the first nucleic acid molecule is not ectopically expressed due to a mutation in an endogenous *TERMINAL FLOWER* gene. For example, the invention provides a method of promoting modulated timing of reproductive development in a seed plant by introducing an ectopically expressible nucleic acid molecule encoding a floral meristem identity gene product into the seed plant, thus producing a transgenic seed plant. A floral meristem identity gene product such as SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, AGL27, AP1, CAL or LFY, or a chimeric protein containing, in part, a floral meristem identity gene product, as disclosed below, is useful in methods of promoting early reproductive development.

The term "promoting early reproductive development," as used herein in reference to a seed plant, means promoting the formation of a reproductive structure earlier than the time when a reproductive structure would form on a corresponding seed plant that is grown under the same conditions and that does not ectopically express a floral meristem identity gene product. As discussed above, the time when reproductive structures form on a particular seed plant that does not ectopically express a floral meristem identity gene product is relatively fixed and depends, in part, on genetic factors as well as environmental conditions, such as day length and temperature. Thus, given a defined set of environmental conditions, a naturally occurring angiosperm, for example, will flower at a relatively fixed time. Similarly, given a defined set of environmental conditions, a naturally occurring coniferous gymnosperm, for example, will produce cones at a relatively fixed time.

Methods for ectopically expressing polynucleotides in plants are well known in the art. For example, the expression of polynucleotides of the invention can be modulated by mutation, or introduction of at least one copy of the polynucleotides into a plant.

One of skill will recognize that a number of methods can be used to modulate gene product activity or gene expression. Gene product activity can be modulated in the plant cell at the gene, transcriptional, posttranscriptional, translational, or posttranslational, levels. Techniques for modulating gene product activity at each of these levels are generally well known to one of skill and are discussed briefly below. "Activity" encompasses both mechanistic activities (e.g., enzymatic, ability to induce transcription of genes under the gene products control, etc.) and phenotypic activities such as altering the time of reproductive development.

Methods for introducing genetic mutations into plant genes are well known. For instance, seeds or other plant material can be treated with a mutagenic chemical substance, according to standard techniques. Such chemical substances include, but are not limited to, the following: diethyl sulfate, ethylene imine, ethyl methanesulfonate and N-nitroso-N-ethylurea. Alternatively, ionizing radiation from sources such as, for example, X-rays or gamma rays can be used.

Alternatively, homologous recombination can be used to induce targeted gene disruptions by specifically deleting or altering the target gene *in vivo* (*see, generally*, Grewal and Klar, *Genetics* 146: 1221-1238 (1997) and Xu *et al.*, *Genes Dev.* 10:2411-2422 (1996)). Homologous recombination has been demonstrated in plants (Puchta *et al.*, *Experientia* 50:277-284 (1994), Swoboda *et al.*, *EMBO J.* 13:484-489 (1994); Offringa *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 7346-7350 (1993); and Kempin *et al. Nature* 389:802-803 (1997)).

In applying homologous recombination technology to the genes of the invention, mutations in selected portions of a gene sequences (including 5' upstream, 3' downstream, and intragenic regions) such as those disclosed here are made *in vitro* and then introduced into the desired plant using standard techniques. Since the efficiency of homologous recombination is known to be dependent on the vectors used, use of dicistronic gene targeting vectors as described by Mountford *et al. Proc. Natl. Acad. Sci. USA* 91:4303-4307 (1994); and Vaulont *et al. Transgenic Res.* 4:247-255 (1995) are conveniently used to increase the efficiency of selecting for altered gene expression in transgenic plants. The mutated gene will interact with the target wild-type gene in such a way that homologous recombination and targeted replacement of the wild-type gene will occur in transgenic plant cells, resulting in suppression of gene product activity.

Alternatively, oligonucleotides composed of a contiguous stretch of RNA and DNA residues in a duplex conformation with double hairpin caps on the ends can be used. The RNA/DNA sequence is designed to align with the sequence of the target gene and to contain the desired nucleotide change. Introduction of the chimeric oligonucleotide on an extrachromosomal T-DNA plasmid results in efficient and specific gene conversion directed by chimeric molecules in a small number of transformed plant cells. This method is described in Cole-Strauss *et al. Science* 273:1386-1389 (1996) and Yoon *et al. Proc. Natl. Acad. Sci. USA* 93:2071-2076 (1996).

Gene expression can be inactivated using recombinant DNA techniques by transforming plant cells with constructs comprising transposons or T-DNA sequences. Mutants prepared by these methods are identified according to standard techniques. For instance, mutants can be detected by PCR or by detecting the presence or absence of mRNA, *e.g.*, by Northern blots. Mutants can also be selected by assaying for altered timing of the development of reproductive structures.

The isolated nucleic acid sequences prepared as described herein, can also be used in a number of techniques to control endogenous gene expression at various levels. Subsequences from the sequences disclosed here can be used to control, transcription, RNA accumulation, translation, and the like.

A number of methods can be used to inhibit gene expression in plants. For instance, antisense technology can be conveniently used. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the antisense strand of RNA will be transcribed. The construct is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been suggested that antisense suppression can act at all levels of gene regulation including suppression of RNA translation (*see*, Bourque *Plant Sci. (Limerick)* 105:125-149 (1995); Pantopoulos In Progress in Nucleic Acid Research and Molecular Biology, Vol. 48. Cohn, W. E. and K. Moldave (Ed.). Academic Press, Inc.: San Diego, California, USA; London, England, UK. p. 181-238; Heiser *et al. Plant Sci. (Shannon)* 127:61-69 (1997)) and by preventing the accumulation of mRNA which encodes the protein of interest, *(see,* Baulcombe *Plant Mol. Bio.* 32:79-88 (1996); Prins and Goldbach *Arch. Virol.* 141:2259-2276 (1996); Metzlaff *et al. Cell* 88:845-854 (1997), Sheehy et al., *Proc. Nat. Acad. Sci. USA*, 85:8805-8809 (1988), and Hiatt et al., U.S. Patent No. 4,801,340).

The nucleic acid segment to be introduced generally will be substantially identical to at least a portion of the endogenous gene or genes to be repressed. The sequence,

however, need not be perfectly identical to inhibit expression. The vectors of the present invention can be designed such that the inhibitory effect applies to other genes within a family of genes exhibiting homology or substantial homology to the target gene.

For antisense suppression, the introduced sequence also need not be full length relative to either the primary transcription product or fully processed mRNA. Generally, higher homology can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 30 or 40 nucleotides and about full length nucleotides should be used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of about 500 to about 7000 nucleotides is especially preferred.

A number of gene regions can be targeted to suppress gene expression. The targets can include, for instance, the coding regions, introns, sequences from exon/intron junctions, 5' or 3' untranslated regions, and the like. In some embodiments, the constructs can be designed to eliminate the ability of regulatory proteins to bind to gene sequences that are required for its cell- and/or tissue-specific expression. Such transcriptional regulatory sequences can be located either 5'-, 3'-, or within the coding region of the gene and can be either promote (positive regulatory element) or repress (negative regulatory element) gene transcription. These sequences can be identified using standard deletion analysis, well known to those of skill in the art. Once the sequences are identified, an antisense construct targeting these sequences is introduced into plants to control gene transcription in particular tissue, for instance, in developing ovules and/or seed. In one embodiment, transgenic plants are selected for activity that is reduced but not eliminated.

Oligonucleotide-based triple-helix formation can be used to disrupt gene expression. Triplex DNA can inhibit DNA transcription and replication, generate site-specific mutations, cleave DNA, and induce homologous recombination (*see, e.g.*, Havre and Glazer *J. Virology* 67:7324-7331 (1993); Scanlon *et al. FASEB J.* 9:1288-1296 (1995); Giovannangeli *et al. Biochemistry* 35:10539-10548 (1996); Chan and Glazer *J. Mol. Medicine (Berlin)* 75:267-282 (1997)). Triple helix DNAs can be used to target the same sequences identified for antisense regulation.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered,

and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. Thus, ribozymes can be used to target the same sequences identified for antisense regulation.

A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Zhao and Pick *Nature* 365:448-451 (1993); Eastham and Ahlering *J. Urology* 156:1186-1188 (1996); Sokol and Murray *Transgenic Res.* 5:363-371 (1996); Sun *et al. Mol. Biotechnology* 7:241-251 (1997); and Haseloff *et al. Nature*, 334:585-591 (1988).

Another method of suppression is sense cosuppression. Introduction of nucleic acid configured in the sense orientation has been recently shown to be an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes (*see,* Assaad *et al. Plant Mol. Bio.* 22:1067-1085 (1993); Flavell *Proc. Natl. Acad. Sci. USA* 91:3490-3496 (1994); Stam *et al. Annals Bot.* 79:3-12 (1997); Napoli et al., *The Plant Cell* 2:279-289 (1990); and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184).

The suppressive effect may occur where the introduced sequence contains no coding sequence *per se*, but only intron or untranslated sequences homologous to sequences present in the primary transcript of the endogenous sequence. The introduced sequence generally will be substantially identical to the endogenous sequence intended to be repressed. This minimal identity will typically be greater than about 65%, but a higher identity might exert a more effective repression of expression of the endogenous sequences. Substantially greater identity of more than about 80% is preferred, though about 95% to absolute identity would be most preferred. As with antisense regulation, the effect should apply to any other proteins within a similar family of genes exhibiting homology or substantial homology.

For sense suppression, the introduced sequence, needing less than absolute identity, also need not be full length, relative to either the primary transcription product or fully processed mRNA. This may be preferred to avoid concurrent production of some plants that are overexpressers. A higher identity in a shorter than full length sequence compensates

for a longer, less identical sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and identity of non-coding segments will be equally effective. Normally, a sequence of the size ranges noted above for antisense regulation is used. In addition, the same gene regions noted for antisense regulation can be targeted using cosuppression technologies.

In a preferred embodiment, expression of a nucleic acid of interest can be suppressed by the simultaneous expression of both sense and antisense constructs (Waterhouse *et al.*, *Proc. Natl. Acad. Sci. USA* 95:13959-13964 (1998). *See also* Tabara *et al. Science* 282:430-431 (1998).

Alternatively, gene product activity may be modulated by eliminating the proteins that are required for cell-specific gene expression. Thus, expression of regulatory proteins and/or the sequences that control gene expression can be modulated using the methods described here.

Another method is use of engineered tRNA suppression of mRNA translation. This method involves the use of suppressor tRNAs to transactivate target genes containing premature stop codons (*see*, Betzner *et al. Plant J.*11:587-595 (1997); and Choisne *et al. Plant J.*11:597-604 (1997). A plant line containing a constitutively expressed gene that contains an amber stop codon is first created. Multiple lines of plants, each containing tRNA suppressor gene constructs under the direction of cell-type specific promoters are also generated. The tRNA gene construct is then crossed into the desired gene product line to activate activity in a targeted manner. These tRNA suppressor lines could also be used to target the expression of any type of gene to the same cell or tissue types.

Proteins may form homogeneous or heterologous complexes *in vivo*. Thus, production of dominant-negative forms of polypeptides that are defective in their abilities to bind to other proteins in the complex is a convenient means to inhibit endogenous gene product activity. This approach involves transformation of plants with constructs encoding mutant polypeptides that form defective complexes and thereby prevent the complex from forming properly. The mutant polypeptide may vary from the naturally occurring sequence at the primary structure level by amino acid substitutions, additions, deletions, and the like. These modifications can be used in a number of combinations to produce the final modified protein chain. Use of dominant negative mutants to inactivate target genes is described in Mizukami *et al. Plant Cell* 8:831-845 (1996).

Another strategy to affect the ability of a protein to interact with itself or with other proteins involves the use of antibodies specific to the protein. In this method cell-

specific expression of specific Abs is used inactivate functional domains through antibody:antigen recognition (*see*, Hupp *et al. Cell* 83:237-245 (1995)).

After plants with reduced activity are identified, a recombinant construct capable of expressing low levels of the gene product can be introduced using the methods discussed below. In this fashion, the level of activity can be regulated to produce preferred plant phenotypes. For example, a relatively weak promoter such as the ubiquitin promoter (see, e.g., Garbarino *et al. Plant Physiol.* 109(4):1371-8 (1995); Christensen *et al Transgenic Res.* 5(3):213-8 (1996); and Holtorf *et al. Plant. Mol. Biol.* 29(4):637-46 (1995)) is useful to produce plants with reduced levels of activity or expression. Such plants are useful for producing, for instance, plants with altered time of developing reproductive structures.

As disclosed herein, ectopic expression of a nucleic acid molecule encoding a floral meristem identity gene product in an angiosperm converts shoot meristem to floral meristem in the angiosperm. Furthermore, ectopic expression of a nucleic acid molecule encoding a floral meristem identity gene product such as AP1, CAL or LFY in an angiosperm prior to the time when endogenous floral meristem identity gene products are expressed in the angiosperm can convert shoot meristem to floral meristem precociously, resulting in early reproductive development in the angiosperm, as indicated by early flowering. In the same manner, ectopic expression of a nucleic acid molecule encoding AP1, CAL, or LFY, for example, in a gymnosperm prior to the time when endogenous floral meristem identity gene products are expressed in the gymnosperm results in early reproductive development in the gymnosperm.

For a given seed plant species and particular set of growth conditions, constitutive expression of a floral meristem identity gene product results in a relatively invariant time of early reproductive development, which is the earliest time when all factors necessary for reproductive development are active. For example, constitutive expression of *AP1* in transgenic *Arabidopsis* plants grown under "long-day" light conditions results in early reproductive development at day 10 as compared to the normal time of reproductive development, which is day 18 in non-transgenic *Arabidopsis* plants grown under the same conditions. Thus, under these conditions, day 10 is the relatively invariant time of early reproductive development for *Arabidopsis* transgenics that constitutively express a floral meristem identity gene product. Similarly, transgenic plants constitutively expressing SEP3 result in plants that develop earlier reproductive structures than wild type plants.

However, in addition to methods of constitutively expressing a floral meristem identity gene product, the present invention provides methods of selecting the time of early

reproductive development. As disclosed herein, floral meristem gene product expression or activity can be regulated in response to an inducing agent or cognate ligand, for example, such that the time of reproductive development can be selected. For example, in *Arabidopsis* transgenics grown under the conditions described above, the time of early reproductive development need not necessarily be the relatively invariant day 10 at which early reproductive development occurs as a consequence of constitutive floral meristem identity gene product expression. If floral meristem identity gene product expression is rendered dependent upon the presence of an inducing agent, early reproductive development can be selected to occur, for example, on day 14, by contacting the seed plant with an inducing agent on or slightly before day 14.

Thus, the present invention provides recombinant nucleic acid molecules, transgenic seed plant containing such recombinant nucleic acid molecules and methods for selecting the time of early reproductive development. These methods allow a farmer or horticulturist, for example, to determine the time of early reproductive development. The methods of the invention can be useful, for example, in allowing a grower to respond to an approaching storm or impending snap-freeze by selecting the time of early reproductive development such that the crop can be harvested before being harmed by the adverse weather conditions. The methods of the invention for selecting the time of early reproductive development also can be useful to spread out the time period over which transgenic seed plants are ready to be harvested. For example, the methods of the invention can be used to increase floral meristem identity gene product expression in different crop fields at different times, resulting in a staggered time of harvest for the different fields.

Thus, the present invention provides a recombinant nucleic acid molecule containing an inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product. The floral meristem identity gene product encoded within a recombinant nucleic acid molecule of the invention can be, for example, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, AGL27, AP1 or CAL. In addition, the floral meristem identity gene product encoded within a recombinant nucleic acid molecule of the invention can be LFY. As disclosed herein, a recombinant nucleic acid molecule of the invention can contain an inducible regulatory element such as a copper inducible element, tetracycline inducible element, ecdysone inducible element or heat shock inducible element.

VII. Inducible Regulatory Elements

The invention also provides a transgenic seed plant containing a recombinant nucleic acid molecule comprising an inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product. Such a transgenic seed plant can be an angiosperm or gymnosperm and can contain, for example, a recombinant nucleic acid molecule comprising an inducible regulatory element operably linked to a nucleic acid molecule encoding AP1 or CAL. Similarly, the ectopic expression of the combination of at least one of AP1 and CALIFLOWER with at least one of SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 can be used to produce seed with various desirable phenotypes. A transgenic seed plant of the invention can contain, for example, a recombinant nucleic acid molecule comprising a copper inducible element, tetracycline inducible element, ecdysone inducible element or heat shock inducible element operably linked to a nucleic acid molecule encoding AP1, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27. In addition, a transgenic seed plant of the invention can contain a recombinant nucleic acid molecule comprising a copper inducible element tetracycline inducible element, ecdysone inducible element or heat shock inducible element operably linked to a nucleic acid molecule encoding CAL. A transgenic seed plant of the invention also can contain a recombinant nucleic acid molecule comprising a copper inducible element, tetracycline inducible element, ecdysone inducible element or heat shock inducible element operably linked to a nucleic acid molecule encoding LFY.

A particularly useful inducible regulatory element can be, for example, a copper-inducible promoter (Mett et al., *Proc. Natl. Acad. Sci. USA* 90:4567-4571 (1993), which is incorporated herein by reference); tetracycline-inducible regulatory element (Gatz et al., *Plant J.* 2:397-404 (1992); Röder et al., *Mol. Gen. Genet.* 243:32-38 (1994), each of which is incorporated herein by reference); ecdysone inducible element (Christopherson et al., *Proc. Natl. Acad. Sci. USA* 89:6314-6318 (1992), which is incorporated herein by reference); or heat shock inducible element (Takahashi et al., *Plant Physiol.* 99:383-390 (1992), which is incorporated herein by reference). Another useful inducible regulatory element can be a lac operon element, which is used in combination with a constitutively expressed lac repressor to confer, for example, IPTG-inducible expression, as described by Wilde et al., (*EMBO J.* 11:1251-1259 (1992), which is incorporated herein by reference).

An inducible regulatory element useful in a method of the invention also can be, for example, a nitrate-inducible promoter derived from the spinach nitrite reductase gene

(Back et al., *Plant Mol. Biol.* 17:9 (1991), which is incorporated herein by reference) or a light-inducible promoter, such as that associated with the small subunit of RuBP carboxylase or the LHCP gene families (Feinbaum et al., *Mol. Gen. Genet.* 226:449 (1991); Lam and Chua, *Science* 248:471 (1990), each of which is incorporated herein by reference). An inducible regulatory element useful in constructing a transgenic seed plant also can be a salicylic acid inducible element (Uknes et al., *Plant Cell* 5:159-169 (1993); Bi et al., *Plant J.* 8:235-245 (1995), each of which is incorporated herein by reference) or a plant hormone-inducible element (Yamaguchi-Shinozaki et al., *Plant Mol. Biol.* 15:905 (1990); Kares et al., *Plant Mol. Biol.* 15:225 (1990), each of which is incorporated herein by reference). A human glucocorticoid response element also is an inducible regulatory element that can confer hormone-dependent gene expression in seed plants (Schena et al., *Proc. Natl. Acad. Sci. USA* 88:10421 (1991), which is incorporated herein by reference).

An inducible regulatory element that is particularly useful for increasing expression of a floral meristem identity gene product in a transgenic seed plant of the invention is a copper inducible regulatory element (see, for example, Mett et al., *supra*, 1993). Thus, the invention provides a recombinant nucleic acid molecule comprising a copper inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product and a transgenic seed plant containing such a recombinant nucleic acid molecule. Copper, which is a natural part of the nutrient environment of a seed plant, can be used to increase expression of a nucleic acid molecule encoding a floral meristem identity gene product operably linked to a copper inducible regulatory element. For example, an ACE1 binding site in conjunction with constitutively expressed yeast ACE1 protein confers copper inducible expression upon an operably linked nucleic acid molecule. The ACE1 protein, a metalloresponsive transcription factor, is activated by copper or silver ions, resulting in increased expression of a nucleic acid molecule operably linked to an ACE1 element.

Such a copper inducible regulatory element can be an ACE1 binding site from the metallothionein gene promoter (SEQ ID NO: 21; Furst et al., *Cell* 55:705-717 (1988), which is incorporated herein by reference). For example, the ACE1 binding site can be combined with the 90 base-pair domain A of the cauliflower mosaic virus 35S promoter and operably linked to a nucleic acid molecule encoding AP1, CAL or LFY to produce a recombinant nucleic acid molecule of the invention. In a transgenic seed plant constitutively expressing ACE1 under control of such a modified CaMV 35S promoter, for example, copper

inducible expression is conferred upon an operably linked nucleic acid molecule encoding a floral meristem identity gene product.

The expression of a nucleic acid encoding a floral meristem identity gene product operably linked to a copper inducible regulatory element, such as 5'-AGCTTAGCGATGCGTCTTTTCCGCTGAACCGTTCCAGCAAAAAAGACTAG-3' (SEQ ID NO: 21), can be increased in a transgenic seed plant grown under copper ion-depleted conditions, for example, and contacted with 50 μM copper sulfate in a nutrient solution or with 0.5 μM copper sulfate applied by foliar spraying of the transgenic seed plant (see, for example, Mett et al., *supra*, 1993). A single application of 0.5 μM copper sulfate can be sufficient to sustain increased floral meristem identity gene product expression over a period of several days. If desired, a transgenic seed plant of the invention also can be contacted with multiple applications of an inducing agent such as copper sulfate.

An inducible regulatory element also can confer tetracycline-dependent floral meristem identity gene expression in a transgenic seed plant of the invention. Thus, the present invention provides a recombinant nucleic acid molecule comprising a tetracycline inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product as well as a transgenic seed plant into which such a recombinant nucleic acid molecule has been introduced. A tetracycline inducible regulatory element is particularly useful for conferring tightly regulated gene expression as indicated by the observation that a phenotype that results from even low amounts of a gene product expression is suppressed from such an inducible system in the absence of inducing agent (see, for example, Röder et al., *supra*, 1994).

A transgenic seed plant constitutively expressing Tn*10*-encoded Tet repressor (TetR), for example, can be contacted with tetracycline to increase expression of a nucleic acid molecule encoding a floral meristem identity gene product operably linked to the cauliflower mosaic virus promoter containing several *tet* operator sequences (5'-ACTCTATCAGTGATAGAGT-3'; SEQ ID NO: 22) positioned close to the TATA box (see, for example, Gatz, *Meth. Cell Biol.* 50:411-424 (1995), which is incorporated herein by reference; Gatz et al., *supra*, 1992). Such a tetracycline-inducible system can increase expression of an operably linked nucleic acid molecule as much as 200 to 500-fold in a transgenic angiosperm or gymnosperm of the invention.

A high level of Tet repressor expression (about 1 x 10^6 molecules per cell) is critical for tight regulation. Thus, a seed plant preferably is transformed first with a plasmid encoding the Tet repressor, and screened for high level expression. For example, plasmid

pBinTet (Gatz, *supra,* 1995) contains the Tet repressor coding region, which is expressed under control of the CaMV 35S promoter, and the neomycin phosphotransferase gene for selection of transformants. To screen transformants for a high level of Tet repressor expression, a plasmid containing a reporter gene under control of a promoter with *tet* operators, such as pTX-Gus-int (Gatz, *supra,* 1995), can be transiently introduced into a seed plant cell and assayed for activity in the presence and absence of tetracycline. High β-glucouronidase (GUS) expression that is dependent on the presence of tetracycline is indicative of high Tet repressor expression.

A particularly useful tetracycline inducible regulatory element is present in plasmid pBIN-HygTX, which has a CaMV 35S promoter, into which three tet operator sites have been inserted, and an octopine synthase polyadenylation site (Gatz, *supra,* 1995). A multiple cloning site between the promoter and polyadenylation signal in pBIN-HygTX allows for convenient insertion of a nucleic acid molecule encoding the desired floral meristem identity gene product, and the hygromycin phosphotransferase gene allows for selection of transformants containing the construct. In a preferred embodiment of the invention, previously selected Tet repressor positive cells are transformed with a plasmid such as pBIN-HygTX, into which a nucleic acid molecule encoding a floral meristem identity gene product has been inserted.

To increase floral meristem identity gene product expression using a tetracycline-inducible regulatory element, a transgenic seed plant of the invention can be contacted with tetracycline or, preferably, with chlor-tetracycline (SIGMA), which is a more efficient inducer than tetracycline. In addition, a useful inducing agent can be a tetracycline analog that binds the Tet repressor to function as an inducer but that does not act as an antibiotic (Gatz, *supra*, 1995). A transgenic seed plant of the invention can be contacted, for example, by watering with about 1 mg/liter chlor-tetracycline or tetracycline. Similarly, a plant grown in hydroponic culture can be contacted with a solution containing about 1 mg/liter chlor-tetracycline or tetracycline (Gatz, *supra,* 1995). If desired, a transgenic angiosperm or gymnosperm can be contacted repeatedly with chlor-tetracycline or tetracycline every other day for about 10 days (Röder et al., *supra*, 1994). Floral meristem identity gene product expression is increased efficiently at a tetracycline concentration that does not inhibit the growth of bacteria, indicating that the use of tetracycline as an inducing agent will not present environmental concerns.

An ecdysone inducible regulatory element also can be useful in practicing the methods of the invention. For example, an ecdysone inducible regulatory element can contain four copies of an ecdysone response element having the sequence 5'-GATCCGACAAGGGTTCAATGCACTTGTCA-3' (EcRE; SEQ ID NO: 23) as described in Christopherson et al., *supra,* 1992. In a transgenic seed plant into which a nucleic acid encoding an ecdysone receptor has been introduced, an ecdysone inducible regulatory element can confer ecdysone-dependent expression on a nucleic acid molecule encoding a floral meristem identity gene product. An appropriate inducing agent for increasing expression of a nucleic acid molecule operably linked to an ecdysone inducible regulatory element can be, for example, ∀-ecdysone, 20-hydroxyecdysone, polypodine B, ponasterone A, muristerone A or RH-5992, which is an ecdysone agonist that mimics 20-hydroxyecdysone (see, for example, Kreutzweiser et al., *Ecotoxicol. Environ. Safety* 28:14-24 (1994), which is incorporated herein by reference and Christopherson et al., *supra,* 1992). Methods for determining an appropriate inducing agent for use with an ecdysone inducible regulatory element are well known in the art. As disclosed herein, compound RH-5992 can be a particularly useful inducing agent for increasing floral meristem gene product expression in a transgenic seed plant containing an ecdysone inducible regulatory element.

An inducible regulatory element also can be derived from the promoter of a heat shock gene, such as *HSP81-1* (SEQ ID NO: 24; Takahashi, *supra*, 1992). Thus, the invention also provides a recombinant nucleic acid molecule comprising a heat shock inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product and a transgenic seed plant containing such a recombinant nucleic acid molecule. The *HSP81-1* promoter (SEQ ID NO: 24) confers low level expression upon an operably linked nucleic acid molecule in parts of roots under unstressed conditions and confers high level expression in most *Arabidopsis* tissues following heat shock (see, for example, Yabe et al., *Plant Cell Physiol.* 35:1207-1219 (1994), which is incorporated herein by reference). After growth of *Arabidopsis* at 23EC, a single heat shock treatment at 37EC for two hours is sufficient to induce expression of a nucleic acid molecule operably linked to the *HSP81-1* gene regulatory element (see Ueda et al., *Mol. Gen. Genet.* 250:533-539 (1996), which is incorporated herein by reference).

The use of a heat shock inducible regulatory element is particularly useful for a transgenic seed plant of the invention grown in an enclosed environment such as a green

house, where temperature can be readily manipulated. The use of a heat shock inducible regulatory element especially is applicable to a transplantable or potted transgenic seed plant of the invention, which can be moved conveniently from an environment having a low temperature to an environment having a high temperature. A transgenic angiosperm or gymnosperm of the invention containing a recombinant nucleic acid molecule comprising a *HSP81-1* heat shock regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product also can be induced, for example, by altering the ambient temperature, watering with heated water or submersing the transgenic seed plant in a sealed plastic bag into a heated water bath (see, for example, Ueda et al., *supra*, 1996).

A recombinant nucleic acid molecule of the invention comprising an inducible gene regulatory element can be expressed variably in different lines of transgenic seed plants. In some transgenic lines, for example, leaky expression of the introduced recombinant nucleic acid molecule can occur in the absence of the appropriate inducing agent due to phenomena such as position effects (see, for example, Ueda et al., *supra*, 1996). Thus, a transgenic seed plant containing a recombinant nucleic acid molecule comprising an inducible gene regulatory element operably linked to a nucleic acid encoding a floral meristem identity gene product can be screened, if desired, to obtain a particular transgenic seed plant in which expression of the operably linked nucleic acid molecule is desirably low in the absence of the appropriate inducing agent.

The present invention also provides a method of converting shoot meristem to floral meristem in an angiosperm by introducing into the angiosperm a recombinant nucleic acid molecule comprising an inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product to produce a transgenic angiosperm, and contacting the transgenic angiosperm with an inducing agent, thereby increasing expression of the floral meristem identity gene product and converting shoot meristem to floral meristem in the transgenic angiosperm. In such a method of the invention, the inducible regulatory element can be, for example, a copper inducible element, tetracycline inducible element, ecdysone inducible element or heat shock inducible element, and the floral meristem identity gene product can be, for example, AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27.

In addition, the invention provides a method of promoting early reproductive development in a seed plant such as an angiosperm or gymnosperm by introducing into the seed plant a recombinant nucleic acid molecule comprising an inducible regulatory element operably linked to a nucleic acid molecule encoding a floral meristem identity gene product

to produce a transgenic seed plant, and contacting the transgenic seed plant with an inducing agent, thereby increasing expression of the floral meristem identity gene product and promoting early reproductive development in the transgenic seed plant. In a method of the invention for promoting early reproductive development in a seed plant, the inducible regulatory element can be, for example, a copper inducible element, tetracycline inducible element, ecdysone inducible element or heat shock inducible element, and the floral meristem identity gene product can be, for example, AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27.

The term "inducing agent," as used herein, means a substance or condition that effects increased expression of a nucleic acid molecule operably linked to a particular inducible regulatory element as compared to the level of expression of the nucleic acid molecule in the absence of the inducing agent. An inducing agent can be, for example, a naturally occurring or synthetic chemical or biological molecule such as a simple or complex organic molecule, a peptide, a protein or an oligonucleotide that increases expression of a nucleic acid molecule operably linked to a particular inducible regulatory element. An example of such an inducing agent is a compound such as copper sulfate, tetracycline or an ecdysone. An inducing agent also can be a condition such as heat of a certain temperature or light of a certain wavelength. When used in reference to a particular inducible regulatory element, an "appropriate" inducing agent means an inducing agent that results in increased expression of a nucleic acid molecule operably linked to the particular inducible regulatory element.

An inducing agent of the invention can be used alone or in solution or can be used in conjunction with an acceptable carrier that can serve to stabilize the inducing agent or to promote absorption of the inducing agent by a seed plant. If desired, a transgenic seed plant of the invention can be contacted with an inducing agent in combination with an unrelated substance such as a plant nutrient, pesticide or insecticide.

One skilled in the art can readily determine the optimum concentration of an inducing agent needed to produce increased expression of a nucleic acid molecule operably linked to an inducible regulatory element in a transgenic seed plant of the invention. For conveniently determining the optimum concentration of inducing agent from a range of useful concentrations, one skilled in the art can operably link the particular inducible regulatory element to a nucleic acid molecule encoding a reporter gene product such as β-glucouronidase (GUS) and assay for reporter gene product activity in the presence of

various concentrations of inducing agent (see, for example, Jefferson et al., *EMBO J.* 6:3901-3907 (1987), which is incorporated herein by reference).

As used herein, the term "contacting," in reference to a transgenic seed plant of the invention, means exposing the transgenic seed plant to an inducing agent, or to a cognate ligand as disclosed below, such that the agent can induce expression of a nucleic acid molecule operably linked to the particular inducible regulatory element. A transgenic seed plant such as an angiosperm or gymnosperm, which contains a recombinant nucleic acid molecule of the invention, can be contacted with an inducing agent in a variety of manners. Expression of a floral meristem identity gene product can be increased conveniently, for example, by spraying a transgenic seed plant with an aqueous solution containing an appropriate inducing agent or by adding an appropriate inducing agent to the water supply of a transgenic seed plant grown using irrigation or to the water supply of a transgenic seed plant grown hydroponically. A transgenic seed plant containing a recombinant nucleic acid molecule of the invention also can be contacted by spraying the seed plant with an inducing agent in aerosol form. In addition, a transgenic seed plant can be contacted with an appropriate inducing agent by adding the agent to the soil or other solid nutrient media in which the seed plant is grown, whereby the inducing agent is absorbed into the seed plant. Other modes of contacting a transgenic seed plant with an inducing agent, such as injecting or immersing the seed plant in a solution containing an inducing agent, are well known in the art. For an inducing agent that is temperature or light, for example, contacting can be effected by altering the temperature or light to which the transgenic seed plant is exposed, or, if desired, by moving the transgenic seed plant from an environment of one temperature or light source to an environment having the appropriate inducing temperature or light source.

If desired, a transgenic seed plant of the invention can be contacted individually with an inducing agent. Furthermore, a group of transgenic seed plants that, for example, are located together in a garden plot, hot house or field, can be contacted *en masse* with an inducing agent, such that floral meristem identity gene product expression is increased coordinately in all transgenic seed plants of the group.

A transgenic seed plant of the invention can be contacted with an inducing agent using one of several means. For example, a transgenic seed plant can be contacted with an inducing agent by non-automated means such as with a hand held spraying apparatus. Such manual means can be useful when the methods of the invention are applied to particularly delicate or valuable seed plant varieties or when it is desirable, for example, to promote early reproductive development in a particular transgenic seed plant without

promoting early reproductive development in a neighboring transgenic seed plant. Furthermore, a transgenic seed plant of the invention can be contacted with an inducing agent by mechanical means such as with a conventional yard "sprinkler" for a transgenic seed plant grown, for example, in a garden; a mechanical spraying system in a green house; traditional farm machinery for spraying field crops; or "crop dusting" for conveniently contacting an entire field of transgenic seed plants with a particulate or gaseous inducing agent. The skilled practitioner, whether home gardener or commercial farmer, recognizes that these and other manual or mechanical means can be used to contact a transgenic seed plant with an inducing agent according to the methods of the invention.

Furthermore, it is recognized that a transgenic seed plant of the invention can be contacted with a single treatment of an inducing agent or, if desired, can be contacted with multiple applications of the inducing agent. In a preferred embodiment of the invention, a transgenic seed plant of the invention is contacted once with an inducing agent to effectively increase floral meristem identity gene product expression, thereby promoting early reproductive development in the transgenic seed plant. Similarly, a transgenic angiosperm of the invention preferably is contacted once with an inducing agent to effectively increase floral meristem identity gene product expression and convert shoot meristem to floral meristem in the transgenic angiosperm.

A single application of an inducing agent is preferable when a transient increase in floral meristem identity gene product expression from a recombinant nucleic acid molecule of the invention promotes irreversible early reproductive development in a seed plant. In many seed plant species, early reproductive development is irreversible. Transient expression of a floral meristem identity gene product from an introduced recombinant nucleic acid molecule, for example, results in sustained ectopic expression of endogenous floral meristem identity gene products, resulting in irreversible early reproductive development. For example, ectopic expression of AP1 in a transgenic plant induces endogenous LFY gene expression, and ectopic expression of LFY induces endogenous AP1 gene expression (Mandel and Yanofsky, *Nature* 377:522-524 (1995), which is incorporated herein by reference; Weigel and Nilsson, *supra,* 1995). Genetic studies also indicate that CAL can act directly or indirectly to increase expression of AP1 and LFY. Thus, ectopic expression of CAL from an exogenous nucleic acid molecule, for example, can induce endogenous AP1 and LFY expression (see Bowman et al., *supra*, 1993). Enhanced expression of endogenous AP1, LFY or CAL following a transient increase in expression of an introduced floral

meristem identity gene product induced by a single application of an inducing agent can make repeated applications of an inducing agent unnecessary.

In some seed plants, however, such as angiosperms characterized by the phenomenon of floral reversion, repeated applications of the inducing agent can be desirable. In species such as impatiens, an initiated flower can revert into a shoot such that the center of the developing flower behaves as an indeterminate shoot (see, for example, Battey and Lyndon, *Ann. Bot.* 61:9-16 (1988), which is incorporated by reference herein). Thus, to prevent floral reversion in species such as impatiens, repeated applications of an inducing agent can be useful. Repeated applications of an inducing agent, as well as single applications, are encompassed within the scope of the present invention.

VIII. Chimeric Polypepides of the Invention

The invention further provides a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding a floral meristem identity gene product such as SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, AGL27, AP1, CAL or LFY linked in frame to a nucleic acid molecule encoding a ligand binding domain. Expression of a chimeric protein of the invention in a seed plant is useful because the ligand binding domain renders the activity of a linked gene product dependent on the presence of cognate ligand. Specifically, in a chimeric protein of the invention, floral meristem gene product activity is increased in the presence of cognate ligand, as compared to activity in the absence of cognate ligand.

A nucleic acid molecule encoding a chimeric protein of the invention comprises a nucleic acid molecule encoding a floral meristem identity gene product, such as a nucleic acid molecule having the nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, which encode AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 and AGL27, respectively, any of which is linked in frame to a nucleic acid molecule encoding a ligand binding domain. The expression of such a nucleic acid molecule results in the production of a chimeric protein containing a floral meristem identity gene product fused to a ligand binding domain. Thus, the invention also provides a chimeric protein containing a floral meristem identity gene product fused to a ligand binding domain and an antibody that specifically binds such a chimeric protein.

The invention further provides a transgenic seed plant, such as angiosperm or gymnosperm, that contains a nucleic acid molecule encoding a chimeric protein of the invention. The invention provides, for example, a transgenic seed plant containing a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding AP1, CAL or LFY linked in frame to a nucleic acid molecule encoding a ligand binding domain. A particularly useful transgenic seed plant contains a nucleic acid molecule encoding AP1 linked in frame to a nucleic acid molecule encoding an ecdysone receptor ligand binding domain or a glucocorticoid receptor ligand binding domain. The invention also provides a transgenic seed plant containing a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding CAL linked in frame to a nucleic acid molecule encoding an ecdysone receptor ligand binding domain or a glucocorticoid receptor ligand binding domain. In addition, there is provided a transgenic seed plant containing a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding LFY linked in frame to a nucleic acid molecule encoding an ecdysone receptor ligand binding domain or a glucocorticoid receptor ligand binding domain.

Any floral meristem identity gene product, as defined herein, is useful in a chimeric protein of the invention. Thus, a nucleic acid molecule encoding *Arabidopsis thaliana* AP1 (SEQ ID NO: 2), *Brassica oleracea* AP1 (SEQ ID NO: 4), *Brassica oleracea* var. *Botrytis* AP1 (SEQ ID NO: 8) or *Zea mays* AP1 (SEQ ID NO: 10), each of which have activity in converting shoot meristem to floral meristem, can be used to construct a nucleic acid molecule encoding a chimeric protein of the invention. Similarly, a nucleic acid molecule encoding, for example, *Arabidopsis thaliana* CAL (SEQ ID NO: 10), *Brassica oleracea* CAL (SEQ ID NO: 12), or a nucleic acid molecule encoding *Arabidopsis thaliana* LFY (SEQ ID NO: 16) is useful when linked in frame to a nucleic acid molecule encoding a ligand binding domain to produce a nucleic acid molecule encoding a ligand-dependent chimeric protein of the invention. Similarly, nucleic acids encoding SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 can be operably linked to a nucleic acid encoding a ligand binding domain.

A ligand binding domain useful in a chimeric protein of the invention is a domain that, when fused in frame to a heterologous gene product, renders the activity of the fused gene product dependent on cognate ligand such that the activity of the fused gene product is increased in the presence of cognate ligand as compared to its activity in the absence of ligand. Such a ligand binding domain can be a steroid binding domain such as the ligand binding domain of an ecdysone receptor, glucocorticoid receptor, estrogen receptor,

progesterone receptor, androgen receptor, thyroid receptor, vitamin D receptor or retinoic acid receptor. A particularly useful ligand binding domain is the ecdysone receptor ligand binding domain contained within amino acids 329 to 878 of the *Drosophila* ecdysone receptor (SEQ ID NO: 18); Koelle et al., *Cell* 67:59-77 (1991); Thummel, *Cell* 83:871-877 (1995), each of which is incorporated herein by reference) or a glucocorticoid receptor ligand binding domain, encompassed, for example, within amino acids 512 to 795 of the rat glucocorticoid receptor (SEQ ID NO: 20; Miesfeld et al., *Cell* 46:389-399 (1986), which is incorporated herein by reference).

A chimeric protein of the invention containing an ecdysone receptor ligand binding domain has floral meristem identity gene product activity that can be increased in the presence of ecdysone ligand. Similarly, a chimeric protein of the invention containing a glucocorticoid receptor ligand binding domain has floral meristem identity gene product activity that is increased in the presence of glucocorticoid ligand. It is well known that in a chimeric protein containing a heterologous gene product such as adenovirus E1A, c-myc, c-fos, the HIV-1 Rev transactivator, MyoD or maize regulatory factor R fused to the rat glucocorticoid receptor ligand binding domain, activity of the fused heterologous gene product can be increased by glucocorticoid ligand (Eilers et al., *Nature* 340:66 (1989); Superti-Furga et al., *Proc. Natl. Acad. Sci., U.S.A.* 88:5114 (1991); Hope et al., *Proc. Natl. Acad. Sci., U.S.A.* 87:7787 (1990); Hollenberg et al., *Proc. Natl. Acad. Sci., U.S.A.* 90:8028 (1993), each of which is incorporated herein by reference).

A nucleic acid molecule encoding a chimeric protein of the invention can be introduced into a seed plant where, under appropriate conditions, the chimeric protein is expressed. In such a transgenic seed plant, floral meristem identity gene product activity can be increased by contacting the transgenic seed plant with cognate ligand. For example, activity of a heterologous protein fused to a rat glucocorticoid receptor ligand binding domain (amino acids 512 to 795) expressed under the control of the constitutive cauliflower mosaic virus 35S promoter in *Arabidopsis* was low in the absence of glucocorticoid ligand; whereas, upon contacting the transformed plants with a synthetic glucocorticoid, dexamethasone, activity of the protein was increased greatly (Lloyd et al., *Science* 266:436-439 (1994), which is incorporated herein by reference). As disclosed herein, a ligand binding domain fused to a floral meristem identity gene product renders the activity of a fused floral meristem identity gene product ligand-dependent such that, upon contacting the transgenic seed plant with cognate ligand, floral meristem identity gene product activity is increased.

Methods for constructing a nucleic acid molecule encoding a chimeric protein of the invention are routine and well known in the art (Sambrook et al., *supra*, 1989). Methods of constructing, for example, a nucleic acid encoding an AP1-glucocorticoid receptor ligand binding domain chimeric protein are described in Example IV of WO 97/46078. For example, the skilled artisan recognizes that a stop codon encoded by the nucleic acid molecule must be removed and that the two nucleic acid molecules must be linked in frame such that the reading frame of the 3' nucleic acid molecule coding sequence is preserved. Methods of transforming a seed plant such as an angiosperm or gymnosperm with a nucleic acid molecule are disclosed above and well known in the art (see Examples I, II and III of WO 97/46078; see, also, Mohoney et al., U.S. Patent Number 5,463,174, and Barry et al., U.S. patent number 5,463,175, each of which is incorporated herein by reference).

As used herein, the term "linked in frame," when used in reference to two nucleic acid molecules that make up a nucleic acid molecule encoding a chimeric protein, means that the two nucleic acid molecules are linked in the correct reading frame such that, under appropriate conditions, a full-length chimeric protein is expressed. In particular, a 5' nucleic acid molecule, which encodes the amino-terminal portion of the chimeric protein, must be linked to a 3' nucleic acid molecule, which encodes the carboxyl-terminal portion of the chimeric protein, such that the carboxyl-terminal portion of the chimeric protein is translated in the correct reading frame. One skilled in the art would recognize that a nucleic acid molecule encoding a chimeric protein of the invention can comprise, for example, a 5' nucleic acid molecule encoding a floral meristem identity gene product linked in frame to a 3' nucleic acid molecule encoding a ligand binding domain or can comprise a 5' nucleic acid molecule encoding a ligand binding domain linked in frame to a 3' nucleic acid molecule encoding a floral meristem identity gene product. Preferably, a nucleic acid molecule encoding a chimeric protein of the invention comprises a 5' nucleic acid molecule encoding a floral meristem identity gene product linked in frame to a 3' nucleic acid molecule encoding a ligand binding domain.

In a transgenic angiosperm containing a chimeric protein of the invention, conversion of shoot meristem to floral meristem can be induced by contacting the transgenic angiosperm with a cognate ligand that is absorbed by the angiosperm and binds the chimeric protein within its ligand binding domain. Thus, the present invention provides a method of converting shoot meristem to floral meristem in an angiosperm by introducing into the angiosperm a nucleic acid molecule encoding a chimeric protein to produce a transgenic angiosperm, where, under appropriate conditions, the chimeric protein containing a floral

meristem identity gene product fused to a ligand binding domain is expressed; and contacting the transgenic angiosperm with cognate ligand, where, upon binding of the cognate ligand to the ligand binding domain, floral meristem identity gene product activity is increased, thereby converting shoot meristem to floral meristem in the transgenic angiosperm.

The present invention provides, for example, a method of converting shoot meristem to floral meristem in an angiosperm by introducing into the angiosperm a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, AGL27, AP1, CAL or LFY linked in frame to a nucleic acid molecule encoding an ecdysone receptor ligand binding domain, to produce a transgenic angiosperm, where, under appropriate conditions, the chimeric protein is expressed; and contacting the transgenic angiosperm with ecdysone ligand, where, upon binding of the ecdysone ligand to the ecdysone receptor ligand binding domain, floral meristem identity gene product activity is increased, thereby converting shoot meristem to floral meristem in the transgenic angiosperm. Similarly, the invention provides, for example, a method of converting shoot meristem to floral meristem in an angiosperm by introducing into the angiosperm a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, AGL27, AP1, CAL or LFY linked in frame to a nucleic acid molecule encoding a glucocorticoid receptor ligand binding domain, to produce a transgenic angiosperm, where, under appropriate conditions, the chimeric protein is expressed; and contacting the transgenic angiosperm with glucocorticoid ligand, where, upon binding of the glucocorticoid ligand to the glucocorticoid receptor ligand binding domain, floral meristem identity gene product activity is increased, thereby converting shoot meristem to floral meristem in the transgenic angiosperm.

In addition, the invention provides a method of promoting early reproductive development in a seed plant by introducing into the seed plant a nucleic acid molecule encoding a chimeric protein of the invention to produce a transgenic seed plant, where, under appropriate conditions, the chimeric protein containing a floral meristem identity gene product fused to a ligand binding domain is expressed; and contacting the transgenic seed plant with cognate ligand, where, upon binding of the cognate ligand to the ligand binding domain, floral meristem identity gene product activity is increased, thereby promoting early reproductive development in the transgenic seed plant. The methods of the invention can be practiced with numerous seed plant varieties. The seed plant can be, for example, an angiosperm such as a cereal plant, leguminous plant, hardwood tree or coffee plant, or can be a gymnosperm such as a pine, fir, spruce or redwood tree.

There is provided, for example, a method of promoting early reproductive development in a seed plant by introducing into the seed plant a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding a floral meristem identity gene product linked in frame to a nucleic acid molecule encoding an ecdysone receptor ligand binding domain, to produce a transgenic seed plant, where, under appropriate conditions, the chimeric protein is expressed; and contacting the transgenic seed plant with ecdysone ligand, where, upon binding of the ecdysone ligand to the ecdysone receptor ligand binding domain, floral meristem identity gene product activity is increased, thereby promoting early reproductive development in the transgenic seed plant. Similarly, the invention provides, for example, a method of promoting early reproductive development in a seed plant by introducing into the seed plant a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 linked in frame to a nucleic acid molecule encoding a glucocorticoid receptor ligand binding domain, to produce a transgenic seed plant, where, under appropriate conditions, the chimeric protein is expressed; and contacting the transgenic seed plant with glucocorticoid ligand, where, upon binding of the glucocorticoid ligand to the glucocorticoid receptor ligand binding domain, floral meristem identity gene product activity is increased, thereby promoting early reproductive development in the transgenic seed plant.

As used herein, the term "ligand" means a naturally occurring or synthetic chemical or biological molecule such as a simple or complex organic molecule, a peptide, a protein or an oligonucleotide that specifically binds a ligand binding domain. In the methods of the present invention, a ligand can be used alone or in solution or can be used in conjunction with an acceptable carrier that can serve to stabilize the ligand or promote absorption of the ligand by a seed plant. If desired, a transgenic seed plant of the invention can be contacted with a ligand for increasing floral meristem identity gene product activity in combination with an unrelated molecule such as a plant nutrient, pesticide or insecticide. When used in reference to a particular ligand binding domain, the term "cognate ligand" means a ligand that, under suitable conditions, specifically binds the particular ligand binding domain.

One skilled in the art readily can determine the optimum concentration of cognate ligand needed to bind a ligand binding domain and increase floral meristem identity gene product activity in a transgenic seed plant of the invention. Generally, a concentration of about 1 nM to 10 μM cognate ligand is useful for increasing floral meristem identity gene

product activity in a transgenic seed plant expressing a chimeric protein of the invention. Preferably, a concentration of about 100 nM to 1 μM cognate ligand is useful for increasing floral meristem identity gene product activity in a transgenic seed plant containing a chimeric protein of the invention (see, for example, Christopherson et al., *Proc. Natl. Acad. Sci. USA* 89:6314-6318 (1992), which is incorporated herein by reference; also, see Lloyd et al., *supra*, 1994). For example, a concentration of about 100 nM to 1 μM dexamethasone can be useful for increasing floral meristem identity gene product activity in a transgenic seed plant of the invention containing a nucleic acid molecule encoding a chimeric protein, which comprises a nucleic acid molecule encoding a floral meristem identity gene product, such as AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 linked in frame to a nucleic acid molecule encoding a glucocorticoid receptor ligand binding domain.

As discussed above, a transgenic seed plant of the invention, such as a transgenic seed plant expressing a chimeric protein of the invention, can be contacted in a variety of manners. A transgenic seed plant can be contacted with cognate ligand, for example, by spraying the seed plant with a gaseous ligand or with solution such as an aqueous solution containing the appropriate ligand; or by adding the cognate ligand to the water supply of a seed plant grown using irrigation or grown hydroponically; or by adding the cognate ligand to the soil or other solid nutrient medium in which a seed plant is grown, whereby the cognate ligand is absorbed into the seed plant to increase floral meristem identity gene product activity. A transgenic seed plant expressing a chimeric protein of the invention also can be contacted with a cognate ligand in aerosol form. In addition, a transgenic seed plant can be contacted with cognate ligand by injecting the seed plant or by immersing the seed plant in a solution containing the cognate ligand.

A transgenic seed plant expressing a chimeric protein of the invention can be contacted individually with cognate ligand, or a group of transgenic seed plants can be contacted *en masse* to increase floral meristem gene product activity synchronously in all seed plants of the group. Furthermore, a variety of means can be used to contact a transgenic seed plant of the invention with cognate ligand to increase floral meristem identity gene product activity. A transgenic seed plant can be contacted with cognate ligand using, for example, a hand held spraying apparatus; conventional yard "sprinkler"; mechanical spraying system, such as an overhead spraying system in a green house; traditional farm machinery, or "crop dusting." As discussed above in regard to the application of inducing agents, the methods of the invention can be practiced using these and other manual or mechanical means to contact a transgenic seed plant with single or multiple applications of cognate ligand.

IX. Nucleic Acid Molecules of the Invention

Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. These techniques and various other techniques are generally performed according to Sambrook *et al.*, *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1989).

The isolation of nucleic acids may be accomplished by a number of techniques. For instance, oligonucleotide probes based on the sequences disclosed here can be used to identify the desired gene in a cDNA or genomic DNA library. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g. using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. To prepare a cDNA library, mRNA is isolated from the desired organ, such as a floral organ, and a cDNA library which contains the gene transcript of interest is prepared from the mRNA. Alternatively, cDNA may be prepared from mRNA extracted from other tissues in which genes of the invention or homologs are expressed.

The cDNA or genomic library can then be screened using a probe based upon the sequence of a cloned nucleic acid disclosed here. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species. Alternatively, antibodies raised against an polypeptide can be used to screen an mRNA expression library.

Alternatively, the nucleic acids of interest can be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of the nucleic acid of the invention directly from genomic DNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. For a general overview of PCR see *PCR Protocols: A Guide to Methods and*

Applications. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), *Academic Press*, San Diego (1990). Appropriate primers and probes for identifying sequences from plant tissues are generated from comparisons of the sequences provided here with other related genes.

The present invention also provides novel substantially purified nucleic acid molecules encoding gene products including AP1, CAL, LFY, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, and AGL27. For example, the invention provides a substantially purified nucleic acid molecule encoding *Brassica oleracea* AP1 having the amino acid sequence SEQ ID NO:4; a substantially purified nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* AP1 having the amino acid sequence SEQ ID NO:6; or a substantially purified nucleic acid molecule encoding *Zea mays* AP1 having the amino acid sequence SEQ ID NO: 8. In addition, the invention provides a substantially purified nucleic acid molecule that encodes a *Brassica oleracea* AP1, *Brassica oleracea* var. *botrytis* AP1 or *Zea mays* AP1 and that contains additional 5' or 3' noncoding sequence. For example, a substantially purified nucleic acid molecule having a nucleotide sequence such as SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 is provided.

The invention also provides a substantially purified nucleic acid molecule encoding a CALIFLOWER gene product such as *Arabidopsis thaliana* CAL (SEQ ID NO: 10) or *Brassica oleracea* CAL (SEQ ID NO: 12). The invention also provides nucleic acid molecules encoding SEP1 (SEQ ID NO:28), SEP2 (SEQ ID NO:30), SEP3 (SEQ ID NO:32), AGL20 (SEQ ID NO:34), AGL22 (SEQ ID NO:36), AGL24 (SEQ ID NO:38) or AGL27 (SEQ ID NO:40).

As used herein in reference to a particular nucleic acid molecule or gene product, the term "substantially purified" means that the particular nucleic acid molecule or gene product is in a form that is relatively free from contaminating lipids, unrelated gene products, unrelated nucleic acids or other cellular material normally associated with the particular nucleic acid molecule or gene product in a cell.

The present invention also provides a nucleotide sequence having at least ten contiguous nucleotides of a nucleic acid molecule encoding any of the above-referenced gene products, including *Brassica oleracea* AP1, *Brassica oleracea* var. *botrytis* AP1 or *Zea mays* AP1, provided that said nucleotide sequence is not present in a nucleic acid molecule encoding a MADS domain containing protein. For example, such a nucleotide sequence can have at least ten contiguous nucleotides of a nucleic acid molecule encoding an AP1 gene product having the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

A nucleotide sequence of the invention can have, for example, at least ten contiguous nucleotides of the nucleic acid sequence of SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7.

As used herein, the term "contiguous," as used in reference to the nucleotides of a nucleic acid molecule means that the nucleotides of the nucleic acid molecule follow continuously in sequence. Thus, a nucleotide sequence of the invention has at least ten contiguous nucleotides of one of the recited nucleic acid molecules without any extraneous intervening nucleotides.

Explicitly excluded from a nucleotide sequence of the present invention is a nucleotide sequence having at least ten contiguous nucleotides that is present in a nucleic acid molecule encoding a MADS domain containing protein. MADS domain containing proteins are well known in the art as described in Purugganan et al., *supra,* 1995.

In general, a nucleotide sequence of the invention can range in size from about 10 nucleotides to the full-length of a cDNA. Such a nucleotide sequence can be chemically synthesized, using routine methods or can be purchased from a commercial source. In addition, such a nucleotide sequence can be obtained by enzymatic methods such as random priming methods, polymerase chain reaction (PCR) methods or by standard restriction endonuclease digestion, followed by denaturation (Sambrook et al., *supra,* 1989).

A nucleotide sequence of the invention can be useful, for example, as a primer for PCR (Innis et al. (ed.) *PCR Protocols: A Guide to Methods and Applications*, San Diego, CA: Academic Press, Inc. (1990)). Such a nucleotide sequence generally contains from about 10 to about 50 nucleotides.

A nucleotide sequence of the invention also can be useful in screening a cDNA or genomic library to obtain a related nucleotide sequence. For example, a cDNA library that is prepared from rice or wheat can be screened with a nucleotide sequence having at least ten contiguous nucleotides of the nucleic acid molecule encoding *Zea mays AP1* (SEQ ID NO: 7) in order to isolate a rice or wheat ortholog of *AP1*. Generally, a nucleotide sequence useful for screening a cDNA or genomic library contains at least about 14 to 16 contiguous nucleotides depending, for example, on the hybridization conditions to be used. A nucleotide sequence containing at least 18 to 20 nucleotides, or containing at least 21 to 25 nucleotides, also can be useful.

A nucleotide sequence having at least ten contiguous nucleotides of a nucleic acid molecule encoding *Zea mays* AP1 (SEQ ID NO: 7) also can be used to screen a *Zea mays* cDNA library to isolate a sequence that is related to but distinct from *AP1*. Similarly, a nucleotide sequence having at least ten contiguous nucleotides of a nucleic acid molecule

encoding *Brassica oleracea* AP1 (SEQ ID NO: 3) or a nucleotide sequence having at least ten contiguous nucleotides of a nucleic acid molecule encoding *Brassica oleracea* var. *botrytis* AP1 (SEQ ID NO: 5) can be used to screen a *Brassica oleracea* or *Brassica oleracea* var. *botrytis* cDNA library to isolate a novel sequence that is related to but distinct from *AP1*. Other gene orthologs, such as of SEP1, SEP2, SEP3, AGL20, AGL22, AGL24 or AGL27 can be isolated by similar methods. In addition, a nucleotide sequence of the invention can be useful in analyzing RNA levels or patterns of expression, as by northern blotting or by *in situ* hybridization to a tissue section. Such a nucleotide sequence also can be used in Southern blot analysis to evaluate gene structure and identify the presence of related gene sequences.

The invention also provides a vector containing a nucleic acid molecule as described above, e.g., encoding a *Brassica oleracea* AP1 gene product, *Brassica oleracea* var. *botrytis* AP1 gene product or *Zea mays* AP1 gene product. A vector can be a cloning vector or an expression vector and provides a means to transfer an exogenous nucleic acid molecule into a host cell, which can be a prokaryotic or eukaryotic cell. Such vectors are well known and include plasmids, phage vectors and viral vectors. Various vectors and methods for introducing such vectors into a cell are described, for example, by Sambrook et al., *supra*, 1989, and by Glick and Thompson, supra, 1993).

The invention further provides a method of producing one of the above-described gene products by expressing a nucleic acid molecule encoding the gene product (e.g., AP1, CAL, SEP1, SEP2, SEP3, AGL20, AGL22, AGL24, or AGL27). Thus, for example, a *Brassica oleracea* AP1 gene product can be produced according to a method of the invention by expressing a nucleic acid molecule having the amino acid sequence of SEQ ID NO: 4 or by expressing a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 3. Similarly, a *Brassica oleracea* var. *botrytis* AP1 gene product can be produced according to a method of the invention by expressing a nucleic acid molecule having the amino acid sequence of SEQ ID NO: 6 or by expressing a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 5. A *Zea mays* AP1 gene product can be produced by expressing a nucleic acid molecule having the amino acid sequence of SEQ ID NO: 8 or by expressing a nucleic acid molecule having the nucleic acid sequence of SEQ ID NO: 7.

The invention also provides a substantially purified AP1 gene product, such as a substantially purified gene product of th invention such as a *Brassica oleracea* AP1 gene product having amino acid sequence SEQ ID NO: 4; a substantially purified *Brassica oleracea* var. *botrytis* AP1 gene product having amino acid sequence SEQ ID NO: 6; or a substantially purified *Zea mays* AP1 gene product having amino acid sequence SEQ ID NO:

8. As used herein, the term "gene product" is used in its broadest sense and includes proteins, polypeptides and peptides, which are related in that each consists of a sequence of amino acids joined by peptide bonds. For convenience, the terms "gene product," "protein" and "polypeptide" are used interchangeably. While no specific attempt is made to distinguish the size limitations of a protein and a peptide, one skilled in the art would understand that proteins generally consist of at least about 50 to 100 amino acids and that peptides generally consist of at least two amino acids up to a few dozen amino acids. The term gene product as used herein includes any such amino acid sequence.

An active fragment of a floral meristem identity gene product also can be useful in the methods of the invention. As used herein, the term "active fragment," means a polypeptide portion of a floral meristem identity gene product that can convert shoot meristem to floral meristem in an angiosperm. An active fragment of an AP1 gene product can consist, for example, of an amino acid sequence that is derived from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and has activity in converting shoot meristem to floral meristem in an angiosperm. An active fragment can be, for example, an amino terminal, carboxyl terminal or internal fragment of *Zea mays* AP1 (SEQ ID NO: 8) that has activity in converting shoot meristem to floral meristem in an angiosperm. The skilled artisan will recognize that an active fragment of a floral meristem identity gene product, as defined herein, can be useful in the methods of the invention for converting shoot meristem to floral meristem in an angiosperm, for producing early reproductive development in a seed plant, or for producing reproductive sterility in a seed plant.

Such an active fragment can be produced using well known recombinant DNA methods (Sambrook et al., *supra*, 1989). Similarly, an active fragment can be, for example, an amino terminal, carboxyl terminal or internal fragment of *Arabidopsis thaliana* CAL (SEQ ID NO: 10) or *Brassica oleracea* CAL (SEQ ID NO: 12) that has activity, for example, in converting shoot meristem to floral meristem in an angiosperm. The product of the *BobCAL* gene (SEQ ID NO: 24), which is truncated at amino acid 150, lacks activity in converting shoot meristem to floral meristem and, therefore, is an example of a polypeptide portion of a CAL floral meristem identity gene product that is not an "active fragment" of a floral meristem identity gene product.

An active fragment of a floral meristem identity gene product, which can convert shoot meristem to floral meristem in an angiosperm, can be identified using the methods described in WO 97/46078. Briefly, an angiosperm such as *Arabidopsis* can be transformed with a nucleic acid molecule encoding a portion of a floral meristem identity

gene product in order to determine whether the portion can convert shoot meristem to floral meristem and, therefore, is an active fragment of a floral meristem identity gene product.

The invention also provides an expression vector containing a nucleic acid molecule encoding a floral meristem identity gene product such as SEP3, AGL20, AGL22, AGL24, AGL27, AP1, CAL or LFY operably linked to a heterologous regulatory element. Expression vectors are well known in the art and provide a means to transfer and express an exogenous nucleic acid molecule into a host cell. Thus, an expression vector contains, for example, transcription start and stop sites such as a TATA sequence and a poly-A signal sequence, as well as a translation start site such as a ribosome binding site and a stop codon, if not present in the coding sequence.

As used herein, the term "heterologous regulatory element" means a regulatory element derived from a different gene than the gene encoding the floral meristem identity gene product to which it is operably linked. A vector containing a floral meristem identity gene, however, contains a nucleic acid molecule encoding a floral meristem identity gene product operably linked to a homolgous regulatory element. Such a vector does not contain a nucleic acid molecule encoding a floral meristem identity gene product operably linked to a heterologous regulatory element and, thus, is not an expression vector of the invention.

The invention further provides a plant expression vector containing a floral meristem identity gene product operably linked to a heterologous regulatory element. For example, a plant expression vector containing a nucleic acid molecule encoding an AP1 gene product having at least about 70 percent amino acid identity with an amino acid sequence of *Arabidopsis thaliana* AP1 (SEQ ID NO: 2) in the region from amino acid 1 to amino acid 163 or with the amino acid sequence of *Zea mays* AP1 (SEQ ID NO: 8) in the region from amino acid 1 to amino acid 163 is provided. A plant expression vector containing a floral meristem identity gene product operably linked to a constitutive regulatory element, such as the cauliflower mosaic virus 35S promoter, is provided. In addition, a plant expression vector containing a floral meristem identity gene product operably linked to an inducible regulatory element is provided.

A useful plant expression vector can contain a constitutive regulatory element for expression of an exogenous nucleic acid molecule in all or most tissues of a seed plant. The use of a constitutive regulatory element can be particularly advantageous because expression from the element is relatively independent of developmentally regulated or tissue-specific factors. For example, the cauliflower mosaic virus 35S promoter (CaMV 35S) is a well-characterized constitutive regulatory element that produces a high level of

expression in all plant tissues (Odell et al., *Nature* 313:810-812 (1985), which is incorporated herein by reference). Furthermore, the CaMV 35S promoter can be particularly useful due to its activity in numerous different seed plant species (Benfey and Chua, *Science* 250:959-966 (1990), which is incorporated herein by reference; Odell et al., *supra*, 1985). Other constitutive regulatory elements useful for expression in a seed plant include, for example, the cauliflower mosaic virus 19S promoter; the Figwort mosaic virus promoter (Singer et al., *Plant Mol. Biol.* 14:433 (1990), which is incorporated herein by reference); and the nopaline synthase (*nos*) gene promoter (An, *Plant Physiol.* 81:86 (1986), which is incorporated herein by reference).

In addition, an expression vector of the invention can contain a regulated gene regulatory element such as a promoter or enhancer element. A particularly useful regulated promoter is a tissue-specific promoter such as the shoot meristem-specific *CDC2* promoter (Hemerly et al., *Plant Cell* 5:1711-1723 (1993), which is incorporated herein by reference), or the *AGL8* promoter, which is active in the apical shoot meristem immediately after the transition to flowering (Mandel and Yanofsky, *supra,* 1995). The promoter of the *SHOOTMERISTEMLESS* gene, which is expressed exclusively in the shoot meristem beginning within an embryo and throughout the angiosperm life cycle, also can be a particularly useful tissue-specific gene regulatory element (see Long et al., *Nature* 379:66-69 (1996), which is incorporated herein by reference).

An appropriate regulatory element such as a promoter is selected depending on the desired pattern or level of expression of a nucleic acid molecule linked thereto. For example, a constitutive promoter, which is active in all tissues, would be appropriate if expression of a gene product in all plant tissues is desired. In addition, a developmentally regulated or tissue-specific regulatory element can be useful to direct floral meristem identity gene expression to specific tissues, for example. As discussed above, inducible expression also can be particularly useful to manipulate the timing of gene expression such that, for example, a population of transgenic seed plants of the invention that contain an expression vector comprising a floral meristem identity gene linked to an inducible regulatory element can undergo early reproductive development at essentially the same time. Selecting the time of reproductive development can be useful, for example, in manipulating the time of crop harvest.

Using nucleic acid molecules encoding gene products provided herein, the skilled artisan can isolate, if desired, novel orthologs. For example, one would choose a region of *AP1* that is highly conserved among known *AP1* sequences such as a region that is

highly conserved between *Arabidopsis AP1* (SEQ ID NO: 1) and *Zea mays AP1* (GenBank accession number L46400; SEQ ID NO: 7) to screen a cDNA or genomic library of interest for a novel AP1 ortholog. One can use a full-length *Arabidopsis AP1* (SEQ ID NO: 1), for example, to isolate a novel ortholog of *AP1* (see, *e.g.*, Example V of WO 97/46078). If desired, the region encoding the MADS domain, which is common to a number of genes, can be excluded, from the sequence used as a probe. Similarly, the skilled artisan knows that a nucleic acid molecule encoding a full-length *CAL* cDNA such as *Arabidopsis* CAL (SEQ ID NO: 9) or *Brassica oleracea* CAL (SEQ ID NO: 11) can be useful in isolating a novel *CAL* ortholog.

For example, the *Arabidopsis AP1* cDNA (SEQ ID NO: 1) can be used as a probe to identify and isolate a novel *AP1* ortholog. Using a nucleotide sequence derived from a conserved region of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, for example, a nucleic acid molecule encoding a novel *AP1* ortholog can be isolated from other plant species. Using methods such as those described by Purugganan et al., *supra,* 1995, one can readily confirm that the newly isolated molecule is an *AP1* ortholog. Thus, a nucleic acid molecule encoding an AP1 gene product, which has at least about 70 percent amino acid identity with the amino acid sequence of SEQ ID NO: 2 (*Arabidopsis* AP1) in the region from amino acid 1 to amino acid 163 or with the amino acid sequence of SEQ ID NO: 8 (*Zea mays* AP1) in the region from amino acid 1 to amino acid 163 can be isolated and identified using well known methods.

Similarly, in order to isolate an ortholog of *CAL*, one can choose a region of *CAL* that is highly conserved among known *CAL* cDNAs, such as a region conserved between *Arabidopsis CAL* (SEQ ID NO: 9) and *Brassica oleracea CAL* (SEQ ID NO: 11). The *Arabidopsis CAL* cDNA (SEQ ID NO: 9) or *Brassica oleracea CAL* cDNA (SEQ ID NO: 11), or a nucleotide fragment thereof, can be used to identify and isolate a novel *CAL* ortholog using methods such as those described in Example V of WO 97/46078. In order to identify related MADS domain genes, a nucleotide sequence derived from the MADS domain of *AP1* or *CAL*, for example, can be useful to isolate a related gene sequence encoding this DNA-binding motif.

Hybridization conditions for isolating a gene ortholog, for example, are relatively stringent such that non-specific hybridization is minimized. Appropriate hybridization conditions can be determined empirically, or can be estimated based, for example, on the relative G+C content of the probe and the number of mismatches between the probe and target sequence, if known. Hybridization conditions can be adjusted as desired

by varying, for example, the temperature of hybridizing or the salt concentration (Sambrook, *supra*, 1989).

The invention also provides a kit for converting shoot meristem to floral meristem in an angiosperm, which contains a plant expression vector having a nucleic acid molecule encoding a floral meristem identity gene product. A kit for promoting early reproductive development in a seed plant, which contains a plant expression vector having a nucleic acid molecule encoding a floral meristem identity gene product, also is provided. If desired, such kits can contain appropriate reagents to facilitate high efficiency transformation of a seed plant with a plant expression vector of the invention. Furthermore, if desired, a control vector lacking a floral meristem identity gene can be included in the kits to determine, for example, the efficiency of transformation.

The following example is offered by way of example, not limitation.

EXAMPLES

Example 1

This example shows the identification of proteins that interact with CAL.

Proteins that interact with CAL

Yeast two-hybrid screens were performed to identify candidate genes whose products interact with AP1 and CAL. The two-hybrid library screens were performed in the YPB2 strain [*MATa ara3 his3 ade2 lys2 trp1 leu2, 112 can^r gal4 gal80 LYS2::GAL1-HIS3, URA3::(GAL1$_{UAS}$*17mers)*-lacZ*]. Yeast were transformed using a modified version of the lithium acetate method of Schiestl and Gietz, *Curr. Genet.* 16, 339-346 (1989).

The two-hybrid cDNA expression library was constructed in the pBI771 (prey) vector using tissue of whole plants at different stages. The bait constructs were prepared by inserting the intact CAL coding region and a truncated form of AP1 into the pBI-880 vector (a variant of pPC62 described in Chevray and Nathans *Proc. Natl. Acad. Sci. USA* 5789-5793 (1992); Kohalmi *et al.*, *Plant. Mol. Biol. Man.* M1, 1-30 (1998)) by inserting the corresponding coding region in-frame at the 3' end of the GAL4 (1-147) sequence contained in the centromere *LEU2* plasmid. These baits tested negative for the ability to activate transcription of both reporters, alone as well as in combination with each the prey vector and an inert control prey, the *Arabidopsis* cruciferin seed storage protein.

SEP3K, SOC1K, SVPK, AGL24K and SOC1KC/2 were generated by polymerase chain reaction (PCR) from the relevant cDNAs using oligos with the appropriate restriction site for posterior cloning into pBI771. The following primers were used:

SEP3-5'K: 5'-CCGTCGACCCATGAGCCAGCAGGAGTATCTC-3'

SEP3-3'Kbox: 5'-CCGCGGCCGCCTTACTCTGAAGATCGTT-3'

SOC1-5'K: 5'-CCGTCGACCCATGAAATATGAAGCAGCAAAC-3'

SOC1-3'Kbox: 5'-CCGCGGCCGCCTCCTTTTGCTTGAGCTG-3'

SOC1-C/2: 5'-CCGCGGCCGCACTTTCTTGATTCTTATT-3'

SVP-5'K: 5'-CCGTCGACCCATGAGTGATCACGCCCGAATG-3'

SVP-3'Kbox: 5'-CCGCGGCCGCTCCCTTTTTCTGAAGTTC-3'

AGL24-5'K: 5'-CCGTCGACCCATGCTTGAGAATTGTAACCTC-3'

AGL24-3'Kbox: 5'-CCGCGGCCGCCTCAAGTGAGAAAATTTG-3'The PCR products were subcloned directly into pCRII (invitrogen) and then digested with SalI-NotI for next subcloning into pBI-771. All constructs were confirmed by sequencing.

CAL screen: The frequency of clones which activated both the *HIS3* and *lacZ* reporters from the 30°C plates was $1/(1.8 \times 10^6) = 5.6 \times 10^{-7}$. The frequency on the 23°C plates was $22/(1.8 \times 10^6) = 1.2 \times 10^{-5}$.

AP1 screen: 9.2×10^4 total transformants were screened at 23°C and the frequency of clones activating both reporter genes was 1.5×10^{-4}.

The transformants were selected on supplemented synthetic dextrose medium lacking leucine, tryptophan and histidine but containing 5 mM 3-amino-1,2,4-triazole. The colonies growing on this selective medium were assayed for β-galactosidase activity on nitrocellulose filters (Kohalmi *et al.*, *supra*). Plasmid DNA from positive clones was isolated and transform into *E. coli*.

Using a full-length CAL cDNA as bait, 23 interacting clones were identified, rescued from yeast and transformed into *E. coli*. Sequence analyses showed that they fell into four classes, all previously identified as *AGAMOUS-like* (*AGL*) genes.

The first class, *SEP3*, included four clones, all of which began within the I-region. Because the cDNA library was poly (T) primed, the clones all comprised varying lengths of the 3' end of the gene. *SEP3* is first expressed in the central dome of stage-two floral primordia and is maintained in the inner three whorls of the flower (Mandel and Yanofsky, *Sex. Plant Reprod.* 11, 22-28 (1998)). *SEP3* acts redundantly with *SEP1* and

SEP2 and is necessary for the development of petals, stamens and carpels (Pelaz *et al.*, *Current Biology* 11, 182-184 (2000)).

The second class identified was the *SUPPRESSOR OF CO OVEREXPRESSION 1* (*SOC1*) gene and included seven clones. The starting point of these clones varied. One clone began with the ATG start codon, another started near the end of the MADS-box, and the remaining clones started at 5' ends of the I-region. *SOC1* is expressed in the inflorescence meristem, as well as in the two inner whorls of the flower beginning in late stage-two and it is involved in promoting flowering (Samach *et al.*, *Science* 288, 1613-1616 (2000)).

The third class was the *SHORT VEGETATIVE PHASE* (*SVP*) gene, and included four clones. Of the clones from this screen, one started in the MADS-box, and three began in the I-region. *SVP* was identified as an *Arabidopsis* expressed sequence tag with homology to the MADS-box family (Alvarez-Buylla *et al.*, *Plant J.* 24, 457-466 (2000)), and it was also cloned by (Hartman *et al.*, 2000) through transposon tagging. *SVP* is a repressor of flowering and is expressed in young leaves and throughout the shoot apical meristem during vegetative development. After the transition to flowering, it is expressed in young flower primordia until stage 3 (Hartman *et al.*, *Plant J.* 21, 351-360 (2000)).

The last eight clones were identified as *AGL24*. One of these clones began within the MADS-box and three within the I-region. In addition, the 5' ends of four clones lie in the first third of the K-box, representing the shortest clones isolated in the screen. *AGL24* was first identified in a previous yeast two-hybrid screen as a clone which interacts with *AG* (Alvarez-Buylla *et al.*, *Proc. Natl. Acad. Sci. USA* 97, 5328-5333 (2000)). *AGL24* is expressed in inflorescences and young floral primordia.

To confirm the specificity of the observed interactions, the longest and shortest clone of each class was transformed back into a yeast strain that contained either the CAL bait, the bait vector, or an inert control bait, cruciferin. The strains containing the CAL bait tested positive for both ß-Gal activity and *HIS* prototrophy. The strains containing the bait vector or the cruciferin bait were negative in both assays, as they were not able to grow on plates lacking histidine and the yeast colonies were completely white in the ß-Gal assay.

AP1 forms dimers in yeast with CAL interactors

The structural and functional similarities between CAL and AP1 suggested that they may interact with an overlapping set of proteins. In order to explore this possibility,

we constructed an AP1 bait by inserting the intact AP1 coding region into the pBI-880 vector. As in the Finley and Brent system, the full-length AP1 bait activated transcription independently. To overcome this problem, a deletion construct was made encoding residues 1-196 of AP1 (AP1Δ1), thus eliminating the putative *trans*-activating C-terminus. In contrast to the full-length AP1 clone, the deletion derivative did not activate the reporter on its own. The longest clone of each class was transformed into yeast in combination with the AP1 deletion bait. In every case, both of the reporters were strongly activated, suggesting that all four CAL-interacting proteins also interact with AP1.

Domain for protein-protein interactions

Previous studies have shown that the MADS-domain and I regions may be important for homodimer formation by AG and by AP1 (Krizek and Meyerowitz, 1996; Mizukami *et al.*, 1996; Riechmann *et al.*, 1996) and that the I region and K-domain are needed for the formation of AP3/PI heterodimers (Krizek and Meyerowitz, *Proc. Natl. Acad. Sci. USA* 93, 4063-4070 (1996); Riechmann *et al.*, *Proc. Natl. Acad. Sci. USA* 93, 4793-4798 (1996)). In addition, the K-domain of AG is sufficient to promote interactions with SEP1, SEP2, SEP3 and AGL6 in yeast (Fan *et al.*, *Plant J.* 11, 999-1010 (1997)). Since many of the CAL- and AP1-interacting clones isolated as part of our study lacked the MADS-domain and I regions, we tested if the K-domain itself was sufficient to promote the observed interactions. First, we subcloned the K-box regions of *SEP3*, *SOC1*, *SVP* and *AGL24* into the prey vector, and tested their ability to activate the reporter using either the empty bait or the cruciferin gene cloned into the bait plasmid. As expected, these K-box regions did not activate the reporter. In contrast, when these K-box prey constructs were introduced into yeast strains that contained each of the CAL or AP1 bait plasmids, reporter activity significantly above background levels was consistently observed. Furthermore, the addition of approximately half of the C-terminal domain of the SOC1 protein was sufficient to greatly strengthen the interaction, similar to what has previously been shown to occur for AG and its interactors (Fan *et al.*, *supra*). Taken together, these studies suggest that the ability of CAL and AP1 to interact with SEP3, SOC1, SVP, and AGL24 is largely mediated by the K-domain. However, other protein domains appear to enhance these interactions since the level of reporter gene activation is higher when larger constructs are used.

Example 2

This example shows the indetifications of proteins that interact with AP1.

Proteins that interact with AP1

In order to find additional proteins that could interact with AP1, the library was screened with the truncated AP1 bait (1-196), and 13 clones that tested positive for ß-Gal activity were characterized. As expected, we found three clones of *AGL20* (also known as *SOC1*), five clones of *AGL22* (also known as *SVP*), and one clone of *AGL24.*

In addition we found one clone of a new MADS box gene designated *AGL27* (Alvarez-Buylla *et al., supra*), two different clones encoding a putative RNA binding protein (GI 10178188), and one clone encoding a novel protein (GI 3157943). We determined that these three newly isolated genes have overlapping expression patterns with that of *AP1*, consistent with the idea that they may interact with AP1 *in planta*.

To confirm the specificity of these interactions, the longest clone of each class was transformed back into yeast with the AP1 bait, the bait vector, and an inert control bait, cruciferin. The strains containing the AP1 bait tested positive for both ß-Gal activity and *HIS* prototrophy. The strains containing the bait vector or the cruciferin bait were negative in both assays. We then tested if the three new AP1-interacting clones could also interact with CAL, since they had not been isolated in the CAL library screen. However, AGL27, the RNA binding protein, and the novel protein were unable to interact with CAL in yeast.

Example 3

This example demonstrates the characteriztion of *sep3* mutants.

sep3* mutants resemble intermediate alleles of *AP1

As a start toward determining if the observed interactions in yeast reflect functional interactions *in vivo*, we characterized loss- and gain-of-function alleles of *SEP3*. If some of the activities of AP1 require an interaction with SEP3, then mutations in *SEP3* might be expected to resemble mutant alleles of *AP1*. We recently identified two independently derived En-1 transposon insertion alleles of *SEP3* and have described the phenotype of *sep1 sep2 sep3* triple mutants in which the three inner whorls of organs become sepaloid (Pelaz *et al., supra*).

The flowers of *sep3-1* and *sep3-2* single mutant plants have petals that are partially transformed into sepals, and infrequently, axillary flowers develop at the base of the first-whorl sepals. When examined by scanning electronic microscopy (SEM), the abaxial cells of these transformed petals resemble cells that are a mixture of abaxial wild type sepal and abaxial wild type petal cells. The abaxial side of the wild type sepals have rectangular cells of varying size, some of which are very long, reaching 300 μm in length. These long cells can be more than ten times the length of the smallest sepal cells. Numerous stomata are visible throughout wild type sepals but are never found on wild-type petals. Cells on the abaxial side of wild type petals all have a uniformly small rounded appearance, and are typically about half of the size of the smallest sepal cells. Unlike wild type petals that have rounded cells, the abaxial side of the *sep3* petals consists of rectangular cells, resembling those found on sepals. Although these mutant petal cells are larger than their wild type counterparts, they are still smaller than the wild type sepal cells. Interestingly, several stomata are interspersed on the surface of these petals, further suggesting a partial transformation of these petals into sepals.

Because the *sep3* petal phenotype resembles that observed for intermediate alleles of *ap1*, (Bowman *et al.*, *Development* 119, 721-743 (1993)), we compared second whorl organs of *sep3* mutants to those of intermediate alleles of *ap1*, including *ap1-2*, *ap1-4* and *ap1-6*. The abaxial cells of these *ap1* mutant petals are very similar to those of the *sep3* mutants, and consist of a blend between petal and sepal cells. These *ap1* mutant cells are larger and more elongated than the wild type petal cells but they do not reach the length of the longer wild type sepal cells. As was observed for *sep3* mutants, petals of these intermediate alleles of *ap1* develop several stomata, further indicating the sepal-like identity. The similarities of *sep* and *ap1* mutants are consistent with the idea that some of the activities of *AP1* are compromised in *sep* mutants, consistent with the possible loss of AP1/SEP interactions.

If the interaction between SEP and AP1 is necessary for AP1 activity, then a reduction in *SEP* expression would be predicted to produce some or all of the *ap1*-mutant phenotypes. To test this idea, we generated transgenic antisense lines in which the 5' end of the *SEP3* gene was expressed in the antisense orientation from the double 35S promoter. Two independent transgenic lines (SP70.1 and SP70.2) were tested for reduction in the amount of *SEP3* mRNA accumulation. As expected, the amount of *SEP3* mRNA in these antisense lines was reduced in comparison to the wild type. The resulting lines underexpressing *SEP3*

showed green petals whose cells appeared partially transformed into sepal cells. These plants also occasionally had axillary flowers arising from the base of the first-whorl sepals. These phenotypes are consistent with a reduction in *AP1* activity, as intermediate alleles of *ap1* produce similar phenotypes. This activity reduction does not mean less *AP1* transcription, the levels of mRNA in these antisense lines are comparable to those of wild type flowers. Interestingly, the green-petal phenotype of these *SEP3* antisense lines is more extreme than that observed for *sep3* single mutants, based on the color change, suggesting that the *SEP3* transgene may also have down regulated other closely related genes such as *SEP1* and *SEP2*.

Example 4

This example demonstrates the characterization of plants overexpressing SEP3.

Constitutive expression of *SEP3*

Previous studies have demonstrated that constitutive expression of *AP1* (35S::AP1) results in plants that flower considerably earlier than wild type plants (Mandel and Yanofsky, *supra*). If some of the activities of *AP1* require an interaction with *SEP3*, as the loss of function studies above would indicate, then it might be expected that constitutive *SEP3* expression would further enhance the 35S::AP1 early-flowering phenotype. To test this hypothesis and to provide further evidence that SEP3 interacts with AP1 *in planta*, we generated 35S::SEP3 sense lines that express constitutively *SEP3* throughout the plant.

Construction of the 35S::SEP3 construct was as follows: cDNA was isolated by RT-PCR using the oligos OAM37: 5'-TAGAAACATCATCTTAAAAAT-3' and SEP3-5': 5'-CCGGATCCAAAATGGGAAGAGGGAGA-3'. This cDNA was first cloned into pCRII (invitrogen) and then digested with BamHI for insertion into the BamHI site of pCGN18 (which contains 35S promoter) to produce sense lines, and confirmed by sequencing. The cDNA cloned into pCRII was digested with BamHI and BglII, the 363bp band corresponding to the 5' end of the cDNA was cloned in antisense orientation into the BamHI site of pBIN-JIT (plasmid carrying two 35S promoters in tandem). The 35S::SEP3 sense and antisense constructs were introduced into *Arabidopsis*, ecotype *Columbia*, by vacuum infiltration (Bechtold *et al.*, *C. R. Acad. Sci.* 316, 1194-1199 (1993)) and transgenic plants were selected on Kanamycin plates.

35S::SEP3 transgenic plants are early flowering, and bolt after producing only four or five rosette leaves, in contrast to wild-type plants which bolt after producing approximately ten leaves under these growth conditions. In addition to the early-flowering phenotype, 35S::SEP3 plants have curled rosette leaves as well as two or three very curled cauline leaves, each of which typically subtends a solitary flower. The primary inflorescence usually produces only a few flowers before terminating. Some of the phenotypes caused by ectopic *SEP3* expression are similar to those conferred by ectopic expression of several other MADS-box genes. However ectopic expression of these other genes often produces additional phenotypes, including alterations in flower organ identity and fruit development that are not seen in the 35S::SEP3 plants.

Example 5

This example demonstrates genetic interactions between 35S::SEP3 and 35S::AP1 transgenes.

To provide genetic evidence that SEP3 and AP1 interact, we crossed the 35S::SEP3 transgene into 35S::AP1 plants. Whereas 35S::AP1 plants flower early after producing four to five rosette leaves, 35S::AP1 35S::SEP3 doubly transgenic plants flower after producing only two rosette leaves, often developing a terminal flower directly from the rosette. Occasionally, these plants produce a very short inflorescence with two cauline leaves that subtend solitary flowers, a terminal flower at the apex, and very little internode elongation. The strong enhancement of the early-flowering phenotypes conferred by each single transgene is consistent with the suggestion that AP1 and SEP3 interact *in planta*.

We also used another genetic approach to investigate the interaction between SEP3 and AP1, avoiding the use of two different transgenic lines. We took advantage of the *tfl1* mutant, in which *AP1* is ectopically activated (Bowman *et al.*, *supra*; Gustafson-Brown *et al.*, *Cell* **76**, 131-143 (1994)), producing a phenotype that closely resembles the 35S::AP1 phenotype. As expected, the *tfl* mutation in combination with the 35S::SEP3 transgene produces the same phenotypes as observed for plants carrying both 35S::AP1 and 35S::SEP3 transgenes. These plants flower after forming two rosette leaves and produce abbreviated shoots with very short internodes and a terminal flower.

Example 6

This example demonstrates the flowering time of an *agl24* mutant.

The effect of *AGL22* (also known as *SVP*) and *AGL24* loss-of-function mutations was assessed. An *agl24* T-DNA insertional mutant (designated W24.2) and an *agl22* mutant (designated svp-E) were obtained and the time to flowering of the mutant plants was measured and compared to wildtype Columbia Arabidopsis plants. On average, the *agl24* mutant produced almost twice as many leaves before flowering than wildtype plants. In addition, the *agl22* mutant produced only half the number of leaves as wildtype before flowering. Results of the experiment, shown in number of leaves prior to flowering, is provided below.

	Rosette	Cauline	Total	N
Columbia	11 +/- 0.9	2.9+/- 0.5	14 +/-1.1	26
svp-E	6+/- 0.6	2.8+/- 0.4	9+/- 0.6	25
W24.2	19+/- 1.5	3.1 +/- 0.5	22 +/- 1.7	26

Thus, the time to flowering and the amount of vegetative growth of the *agl24* mutant was increased compared to wild type plants and the time to flowering and the amount of vegetative growth of the *agl22* mutant was decreased compared to wild type plants.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, databases, Genbank sequences, patents, and patent applications cited herein are hereby incorporated by reference.